Electronic Supplementary Material (ESI) for Polymer Chemistry.

Characterization of a library of vitamin A-functionalized polymethacrylate-based nanoparticles for siRNA delivery

Supporting information

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Materials

Methyl methacrylate (MMA, stabilized, Sigma Aldrich, 99%), (2-dimethylaminoethyl) methacrylate (DMAEMA, stabilized, Sigma Aldrich, 98%), poly(ethylene glycol) methacrylate M_n = 360 g mol^{-1} ($PEGMA_{360}$, stabilized, Sigma Aldrich) and poly(ethylene glycol) methacrylate M_n = 500 g mol^{-1} ($PEGMA_{500}$, stabilized, Sigma Aldrich) were used after removing the inhibiting monomethyl ether hydroquinone (MEHG) using the inhibitor remover for removing hydroquinone and monomethyl ether hydroquinone (Sigma Aldrich). 2,2'-Azobis(2-methylpropionitrile) (AIBN, Sigma Aldrich, 98%), 2-cyano-2-propyl benzodithioate (CPDB, Strem Chemicals, 97%), 1,3,5-trioxane (Sigma Aldrich, 99%), *all-trans*-retinoic acid (RA, TCI, 93%), *N*,*N*'-dicyclohexylcarbodiimide (DCC, Sigma Aldrich, 99%), 4-(dimethylamino)pyridine (DMAP, Sigma Aldrich, 98%), anhydrous dimethyl sulfoxide (DMSO, Sigma Aldrich, 99.9%), poly(vinyl alcohol) (Mowiol 4-88, Sigma Aldrich), tRNA (from brewer's yeast, Sigma Aldrich), siRNA and labeled siRNA (Eurofins Genomics) were used as received. Bio-Beads (S-X1, Bio Rad) were utilized according to the manufacturer's protocol using freshly distilled tetrahydrofuran (THF) as the eluent. The anhydrous solvents CH_2Cl_2 and THF were dried in a solvent purification system prior to use (SPS, Pure solv. EN, InnovativeTechnology). All other solvents were purchased from standard suppliers and were used without any further purification. Agarose (HR-Plus), ethidium bromide solution (1%) and glycine were purchased from Carl Roth. DNA-Ladder (100 BP) and Gel Loading Buffer (6x Green) were purchased from Jena Bioscience. Heparin sodium salt was purchased from Alfa Aesar. RNase A (10 mg/mL) and RNase free water (UltraPure^{TM}) were purchased from Thermo Fisher Scientific and Bromophenol Blue from ACROS Organics^{TM}. Glycerol, TRIS, acetate-, citrate-, and phosphate buffer were purchased from Sigma-Aldrich. Coomassie Brilliant Blue R-250 solution was purchased from G Biosciences. Pre-casts PAGE gels (Mini-PROTEAN 4-15%) were purchased from Bio Rad. Hydrochloric acid (AnalaR® NORMAPUR®) was purchased from VWR. The retinol-binding protein (RBP) was purchased from MyBioSource,

Inc. The bovine serum albumin (BSA, 98%) was purchased from Sigma Aldrich. The 0.01 M phosphate buffered saline (PBS) and the buffer solution (pH 7.4) were purchased from Sigma Aldrich.

Detailed amounts of reactants used for polymer synthesis

Table S1: Summary for P(MMA-*stat*-DMAEMA) copolymers **Ci** representing the hydrophobic blocks.

#	MMA		DMAEMA		AIBN (I)		CPDB (CTA)		[M]:[CTA]:[I]	DMF	$\%_{conv.}$	Yield	
	m	n	m	n	m	n	m	n		V			
	g	mmol	g	mmol	mg	mmol	g	mmol		mL	%	g	%
C1	4.00	39.95			11.48	0.07	61.90	0.28	143:1:0.25	6.579	82	2.57	64
C2	4.00	40.0			11.48	0.07	61.90	0.28	143:1:0.25	5.627	70	1.63	41
C3	4.00	40.0			11.48	0.07	61.90	0.28	143:1:0.25	7.414	67	1.63	52
C4	4.00	40.0	0.13	0.8	16.40	0.10	88.43	0.40	102:1:0.25	6.813	90	2.48	78
C5	4.00	40.0	0.38	2.4	16.40	0.10	88.43	0.40	106:1:0.25	7.051	91	2.42	55
C6	4.00	40.0	0.63	4.0	11.48	0.07	61.90	0.28	157:1:0.25	8.087	66	2.34	51
C7	4.00	40.0	0.63	4.0	11.48	0.07	61.90	0.28	157:1:0.25	7.252	83	3.17	68
C8	3.20	32.0	1.26	8.0	11.50	0.07	61.98	0.28	143:1:0.25	7.722	61	2.70	61
C9	2.80	28.0	1.89	12.0	11.50	0.07	61.97	0.28	143:1:0.25	7.172	70	2.89	62
C10	10.51	105.0	7.07	45.0	43.11	0.26	232.42	1.05	143:1:0.25	32.303	64	9.79	56
C11	2.40	24.0	2.52	16.0	11.49	0.07	61.97	0.28	143:1:0.25	7.42	71	2.90	59
C12	9.01	90.0	9.43	60.0	43.10	0.26	232.39	1.05	143:1:0.25	23.497	63	9.67	52
C13	2.00	20.0	3.14	20.0	11.50	0.07	61.99	0.28	143:1:0.25	7.669	71	2.92	57

Table S2: Summary for P(MMA-*stat*-DMAEMA)-*b*-PPEGMA block copolymers **Pi**.

#	$PEGMA_{360}$		$PEGMA_{500}$		AIBN		macroCTA		[M]:[macroCTA]:[I]	DMF	$\%_{conv.}$	Yield	
	m	n	m	n	m	n	m	n		V			
	mg	mmol	mg	mmol	mg	mmol	mg	mmol		mL	%	mg	%
P1	185	0.51			0.21	0.013	**C3**	501	0.051	10:1:0.25	4.965	48	452	66
P2	454	1.26			2.59	0.016	**C1**	755	0.063	20:1:0.25	10.125	85	653	54
P3			120	0.24	0.99	0.006	**C3**	289	0.024	10:1:0.25	4.901	53	222	54
P4			295	0.59	1.21	0.007	**C1**	301	0.030	20:1:0.25	5.605	62	236	40
P5	189	0.53			2.16	0.013	**C4**	500	0.053	10:1:0.25	5.084	58	437	69
P6	573	1.59			3.28	0.020	**C4**	759	0.080	20:1:0.25	10.158	90	533	40
P7			162	0.32	1.33	0.008	**C4**	309	0.033	10:1:0.25	6.158	46	260	55
P8			317	0.63	1.30	0.008	**C4**	302	0.032	20:1:0.25	6.017	60	375	61
P9	177	0.49			2.02	0.012	**C5**	501	0.049	10:1:0.25	4.746	67	410	60
P10	534	1.48			3.03	0.018	**C5**	751	0.074	20:1:0.25	10.147	89	822	64
P11			151	0.30	1.24	0.008	**C5**	307	0.030	10:1:0.25	5.751	35	231	50
P12			304	0.61	1.25	0.008	**C5**	310	0.030	20:1:0.25	5.611	62	360	59
P13	129	0.36			1.47	0.009	**C7**	500	0.036	10:1:0.25	3.463	59	336	53
P14	340	1.08			2.22	0.014	**C7**	755	0.054	20:1:0.25	10.107	83	974	89

P15			135	0.27	1.11	0.007	**C6**	301	0.027	10:1:0.25	5.251	51	225	52
P16			272	0.55	1.12	0.007	**C6**	304	0.027	20:1:0.25	5.127	58	331	57
P17	227	0.63			2.58	0.018	**C8**	623	0.063	10:1:0.25	3.835	69	327	38
P18	457	1.27			2.60	0.016	**C8**	628	0.063	20:1:0.25	7.668	62	512	47
P19	327	0.91			3.73	0.023	**C10**	997	0.091	10:1:0.25	5.781	51	804	61
P20	183	0.51			2.09	0.013	**C9**	604	0.051	10:1:0.25	3.206	65	516	66
P21	687	1.91			3.92	0.024	**C10**	1047	0.095	20:1:0.25	11.713	48	1146	66
P22	371	1.03			2.12	0.013	**C9**	611	0.052	20:1:0.25	6.428	63	813	83
P23			467	0.93	3.83	0.023	**C10**	1024	0.093	10:1:0.25	5.654	68	618	41
P24			917	1.83	3.77	0.023	**C10**	1006	0.092	20:1:0.25	11.322	67	433	23
P25	171	0.48			1.95	0.012	**C11**	605	0.048	10:1:0.25	2.981	68	589	76
P26	347	0.96			1.98	0.012	**C11**	614	0.048	20:1:0.25	5.858	59	663	69
P27			444	0.89	3.64	0.022	**C12**	1007	0.089	10:1:0.25	5.471	59	512	35
P28			896	1.79	3.68	0.022	**C12**	1017	0.090	20:1:0.25	10.934	61	495	26
P29	167	0.47			1.91	0.012	**C13**	616	0.047	10:1:0.25	2.871	63	659	84
P30	327	0.91			1.87	0.011	**C13**	601	0.045	20:1:0.25	5.750	67	899	77

Table S3: Summary for Vit. A functionalized block copolymers **Pi***.

#		P		RA		DCC		DMAP		CH_2Cl_2	THF	Yield	
	Precursor	m	n	m	n	m	n	m	n	V	V		
		mg	mmol	mg	mmol	mg	mmol	mg	mmol	mL	mL	mg	%
P1*	**P1**	158	0.014	30.33	0.10	24.19	0.12	14.85	0.12	5.58	0.42	121	68
P2*	**P2**	154	0.022	67.48	0.22	56.21	0.27	32.44	0.27	5.58	0.42	155	78
P3*	**P3**	200	0.018	32.78	0.11	26.48	0.13	15.79	0.13	5.68	0.32	198	89
P4*	**P 4**	199	0.012	70.81	0.24	56.70	0.27	34.46	0.28	5.52	0.48	180	73
P5*	**P5**	156	0.013	35.19	0.12	29.57	0.14	11.88	0.10	5.52	0.48	122	68
P6*	**P6**	154	0.009	74.23	0.25	61.32	0.30	36.04	0.30	6.54	0.46	136	67
P7*	**P7**	207	0.018	36.23	0.12	29.79	0.14	17.54	0.14	5.65	0.35	189	82
P8*	**P8**	202	0.013	34.18	0.11	28.35	0.14	15.66	0.13	5.77	0.23	173	77
P9*	**P9**	150	0.012	36.40	0.12	29.57	0.14	17.82	0.15	5.50	0.50	118	68
P10*	**P10**	155	0.010	78.28	0.26	63.88	0.31	39.64	0.32	6.52	0.48	132	64
P11*	**P11**	202	0.017	27.60	0.09	23.17	0.11	14.03	0.12	5.77	0.23	171	78
P12*	**P12**	199	0.012	68.37	0.23	56.70	0.27	31.33	0.26	6.53	0.47	156	64
P13*	**P13**	154	0.010	25.48	0.08	20.16	0.10	11.88	0.10	5.65	0.35	117	68
P14*	**P14**	150	0.008	56.68	0.19	48.55	0.24	28.83	0.24	5.65	0.35	62	33

P15*	**P15**	201	0.015	34.50	0.11	28.14	0.14	17.54	0.14	5.67	0.33	154	68
P16*	**P16**	205	0.012	31.74	0.11	26.33	0.13	15.66	0.13	5.78	0.22	130	57
P17*	**P17**	176	0.013	39.62	0.13	29.42	0.14	19.63	0.16	5.80	0.20	135	67
P18*	**P18**	179	0.011	72.60	0.24	58.97	0.29	35.34	0.29	5.63	0.37	142	62
P19*	**P19**	502	0.039	45.84	0.15	39.28	0.19	23.91	0.20	9.73	0.27	398	74
P20*	**P20**	169	0.012	36.34	0.12	30.93	0.15	16.54	0.14	5.75	0.25	124	65
P21*	**P21**	529	0.037	80.22	0.27	68.74	0.33	39.85	0.33	9.53	0.47	272	46
P22*	**P22**	174	0.011	60.56	0.20	49.48	0.24	28.95	0.24	5.58	0.42	130	61
P23*	**P23**	319	0.024	35.97	0.12	30.68	0.15	18.74	0.15	7.63	0.37	222	64
P24*	**P24**	228	0.015	42.51	0.14	34.52	0.17	21.42	0.18	5.57	0.43	111	43
P25*	**P25**	174	0.011	36.34	0.12	30.96	0.15	16.54	0.15	5.75	0.25	125	63
P26*	**P26**	177	0.010	58.14	0.19	46.39	0.22	28.95	0.24	5.60	0.40	130	60
P27*	**P27**	230	0.017	22.20	0.07	18.22	0.09	10.07	0.08	5.70	0.30	68	28
P28*	**P28**	219	0.014	38.23	0.13	31.89	0.15	20.13	0.16	5.48	0.52	95	38
P29*	**P29**	179	0.012	33.92	0.11	27.83	0.13	16.54	0.14	5.77	0.23	145	72
P30*	**P30**	176	0.010	60.56	0.21	49.48	0.24	28.95	0.24	5.58	0.42	126	58

Summarized characteristics of polymer library

Table S4: Overview of characterization data of PMMA and P(MMA-*stat*-DMAEMA) polymers **C1** to **C13** used as hydrophobic core.

#	DMAEMA[a)] mol%	DMAEMA[b)] mol%	DP_{total}[b)]	$M_{n.theo}$[c)] g mol^{-1}	M_n[d)] g mol^{-1}	Đ[d)]	macroCTA for
C1	0	0	117	12,000	13,400	1.19	**P2, P3**
C2	0	0	100	10,200	11,900	1.19	**P4**
C3	0	0	95	9,700	11,500	1.21	**P1**
C4	2	2	92	9,500	11,200	1.23	**P5-P8**
C5	6	6	96	10,200	11,800	1.21	**P9-P12**
C6	9	9	104	11,200	12,900	1.25	**P15, P16**
C7	9	9	131	14,000	15,100	1.23	**P13, P14**
C8	21	20	87	9,900	10,200	1.25	**P17, P18**
C9	31	30	99	11,900	12,600	1.22	**P20, P22**
C10	31	30	92	11,000	9,100	1.36	**P19, P21, P23, P24**
C11	42	40	102	12,800	13,200	1.22	**P25, P26**
C12	42	40	91	11,300	10,900	1.36	**P27, P28**
C13	51	50	101	13,200	14,000	1.23	**P29, P30**

a) Calculated by integration of ^{1}H-NMR signals assigned to the methyl ester group of MMA repeating units relative to the methylene signals adjacent to the ester moiety of DMAEMA repeating units in the ^{1}H NMR spectra of the purified polymers. b) Calculated based on conversion via ^{1}H-NMR spectroscopy (**Equations S1** and **S2**). c) Calculated based on ^{1}H-NMR spectroscopy (**Equation S4**). d) Determined by SEC ($CHCl_3$ 94%, triethylamine 4%, 2-propanol 2%, RI detection, PMMA calibration).

Table S5: Overview of characterization data of P(MMA-*stat*-DMAEMA)-*b*-PPEGMA **P1** to **P30**, the retinoic acid functionalized P(MMA-*stat*-DMAEMA)-*b*-P(PEGMA-*stat*- PEGMA-RA) polymers **P1*** to **P30*** and the resulting nanoparticle formulation that are loaded with tRNA.

#	PEGMA[a)]	DP_{PEGMA}[b)]		DP_{Total}[c)]	$M_{n,theo}$[d)]	M_n[e)]	Đ[e)]	Dh[f)]	PDI[f)]	ζ[f)]	EE[g)]	# RA	DF_{RA}	Dh[f)]	PDI[f)]	ζ[f)]	EE[g)]
	mol%	360 g mol^{-1}	500 g mol^{-1}		g mol^{-1}	g mol^{-1}		nm		mV	%		%	nm		mV	%
P1	6	5		105	18,100	32,500	1.44	125	0.17	-6.16	44	**P1***	11	119[h)]	0.27[h)]	-2.15[h)]	
P2	18	17		131	11,500	15,600	1.18	178	0.27	-1.99	0	**P2***	27	-	-	-	
P3	4		5	100	16,400	20,000	1.21	121	0.12	-3.89	44	**P3***	17	96[h)]	0.15[h)]	2.67[h)]	
P4	11		12	129	14,600	17,400	1.16	112	0.17	-6.45	30	**P4***	17	104[h)]	0.27[h)]	-22[h)]	
P5	5	6		98	11,600	16,400	1.19	143	0.16	-5.11	49	**P5***	15	169[h)]	0.31[h)]	-4.15[h)]	
P6	9	18		110	16,600	40,300	1.42	206	0.30	1.42	50	**P6***	-	-	-	-	
P7	2		5	97	11,800	16,300	1.17	133	0.21	-4.38	18	**P7***	12	103[h)]	0.19[h)]	-3.83[h)]	
P8	6		12	104	15,500	21,000	1.21	134	0.22	2.90	59	**P8***	11	130[h)]	0.16[h]	-4.12[h)]	
P9	6	7		103	12,600	17,100	1.18	127	0.22	12	37	**P9***	17	132[h)]	0.15[h)]	-1.13[h)]	
P10	11	18		114	16,000	39,100	1.50	175	0.30	5.48	98	**P10***	-	-	-	-	
P11	5		4	100	11,800	16,900	1.17	134	0.16	10.51	90	**P11***	10	102[h)]	0.20[h)]	-4.78[h)]	
P12	9		12	108	16,200	22,200	1.21	105	0.26	0.09	54	**P12***	24	159[h)]	0.25[h)]	1.45[h)]	
P13	2	6		139	16,100	20,300	1.19	120	0.17	-5.13	72	**P13***	13	184[h)]	0.38[h)]	-2.48[h)]	
P14	24	17		148	20,000	40,300	1.42	178	0.35	7.96	89	**P14***	-	-	-	-	

	PEGMA[a]	$DP_{PEGMA360}$[b]	$DP_{PEGMA500}$[b]	DP_{Total}[c]	$M_{n,theo}$[d]	M_n[e]	Đ[e]	Dh[f]	PDI[f]	ζ[f]	EE[g]	# RA	DF_{RA}	Dh[f]	PDI[f]	ζ[f]	EE[g]
	mol%				g mol^{-1}	g mol^{-1}		nm		mV	%		%	nm		mV	%
P15	5		5	109	13,700	17,300	1.23	140	0.23	18.2	97	**P15***	19	89[h]	0.15[h]	-1.43[h]	
P16	6		12	116	17,000	19,700	1.36	96	0.16	-3.46	59	**P16***	17	107[h]	0.20[h]	7.33[h]	
P17	7	7		94	13,900	16,200	1.26	148	0.24	4.17	75	**P17***	23	117[h]	0.28[h]	-17.7[h]	
P18	15	12		99	15,800	17,800	1.24	58	0.21	0.16	93	**P18***	-	-	-	-	
P19	1	5		97	12,800	12,100	1.38	92	0.29	1.65	99	**P19***	9	70	0.39	6.86	99
P20	7	6		105	14,200	18,600	1.24	112[h]	0.22[h]	4.38	82	**P20***	29	85[h]	0.12[h]	-4.12[h]	
P21	5	10		102	14,400	13,900	1.35	80	0.26	2.68	98	**P21***	10	67	0.25	-0.10	99
P22	10	13		112	16,400	19,200	1.27	83	0.21	-3.82	85	**P22***	-	-	-	-	
P23	2		7	97	13,400	13,900	1.57	75	0.20	12.90	99	**P23***	10	97	0.26	3.48	99
P24	5		13	105	15,800	18,900	1.65	81	0.36	2.85	98	**P24***	5	67	0.27	1.54	99
P25	3	7		109	15,200	17,600	1.30	92	0.26	2.82	86	**P25***	28	59[h]	0.37[h]	-1.66[h]	
P26	4	13		115	17,000	19,000	1.29	77	0.22	-1.09	98	**P26***	-	-	-	-	
P27	5		6	97	13,500	19,400	1.47	74	0.19	-2.10	99	**P27***	7	63	0.18	-0.97	99
P28	7		12	103	15,800	19,600	1.45	174	0.30	11.06	99	**P28***	5	72	0.35	1.43	99
P29	2	6		108	15,500	18,700	1.31	91	0.40	3.75	94	**P29***	13	89[h]	0.26[h]	-0.77[h]	
P30	5	13		113	18,100	20,100	1.30	67	0.26	-3.96	99	**P30***	-	-	-	-	

a) Calculated by integration from ^{1}H-NMR signal of methylene group of tertial aminogroup of DMAEMA relative to overlapping signal of ester methylene groups of DMAEMA and PEGMA. b) Calculated based on conversion via ^{1}H-NMR spectroscopy (**Equation S1** and **S2**). c) Total DP including hydrophobic block. d) Calculated molar mass based on ^{1}H-NMR spectroscopy (**Equation S4** and **S5**). e) Determined by SEC ($CHCl_3$ 94%, triethylamine 4%, 2-propanol 2%, RI detection, PMMA calibration). f) Hydrodynamic diameter (Dh) of tRNA loaded nanoparticles by DLS measurement. g) tRNA quantification via Quantifluor assay. h) Characterization of water loaded nanoparticles by DLS measurements.

Calculation of conversion via ^{1}H-NMR spectroscopy via and the internal standard trioxane

$$\%_{conv} = \left(1 - \frac{I_{vinyl,end} / I_{trioxane,end}}{I_{vinyl,0} / I_{trioxane,0}} \right) \cdot 100\% \quad \text{(S1)}$$

The resulting overall degree of polymerization (DP) was calculated using $\%_{conv}$ and [M]/[CTA]:

$$DP = \%_{conv} \cdot [M]/[CTA] \quad \text{(S2)}$$

The DP of MMA and DMAEMA ($DP_{MMA/DMAEMA}$) were estimated according to the feed ratio of the monomers ($\%_{MMA/DMAEMA}$):

$$DP_{MMA/DMAEMA} = DP \cdot \%_{MMA/DMAEMA} \quad \text{(S3)}$$

Based on the conversion, the molar mass $M_{n,theo,Ci}$ of each polymer was calculated using the following equation:

$$M_{n,theo,Ci} = DP_{MMA} \cdot M_{MMA} + DP_{DMAEMA} \cdot M_{DMAEMA} + M_{CDPB} = M_{n,theo,macroCTA} \quad \text{(S4)}$$

Based on the conversion, the molar mass of each block copolymer P_i was calculated using the following equation.

$$M_{n,theo\ Pi} = DP_{PEGMA} \cdot M_{n,PEGMA} + M_{n,theo,macroCTA} \quad \text{(S5)}$$

Assignments of signals in ^{1}H-NMR spectra to polymer structure

Bold numbers refer to the peak assignments as depicted in **Figure 1**.

C2: ^{1}H-NMR ($CDCl_3$, 300 MHz, 298 K): δ = 3.60 (3.0H, **3**), 2.14 - 1.72 (1.9H, **1**). 1.52 - 0.71 (3.3H, **2**) ppm.

C9: ^{1}H-NMR ($CDCl_3$, 300 MHz, 298 K): δ = 4.07 (0.9H, **4**), 3.60 (3.0H, **3**), 2.56 (0.9H, **5**), 2.28 (2.7H, **6**), 2.14 - 1.72 (2.8H, **1/1'**), 1.52 - 0.71 (7.2H, **2/2'**) ppm.

P20: ^{1}H-NMR ($CDCl_3$, 300 MHz, 298 K): δ = 4.07 (1.2H, **4**), 3.67 – 3.50 (5.3H, **3**, **7**), 2.56 (0.9H, **5**), 2.28 (2.8H, **6**; **8**), 2.14 - 1.72 (3.2H, **1/1'/1''**), 1.52 - 0.71 (6.0H, **2/2'/2''**) ppm.

P20*: ^{1}H-NMR ($CDCl_3$, 300 MHz, 298 K): δ = 7.21 - 5.72 (0.2H, **9**, **11**, **12**, **13**, **15**, **16**), 4.07 (0.9H, **4**), 3.67 - 3.44 (4.6H, **3**, **7**), 2.56 (0.8H, **5**), 2.28 (2.3H, **6**, **8**), 2.14 - 1.72 (4.7H, **1/1'/1''**, **10**, **14**, **22**, **19**, **20**, **21**), 1.52 - 0.71 (5.8H, **2/2'/2''**) ppm.

*SEC elugrams of P(MMA-stat-DMAEMA) macroCTA **C1-C13** in comparison to P(MMA-stat-DMAEMA)-b-PPEGMA block copolymers **P1-P30***

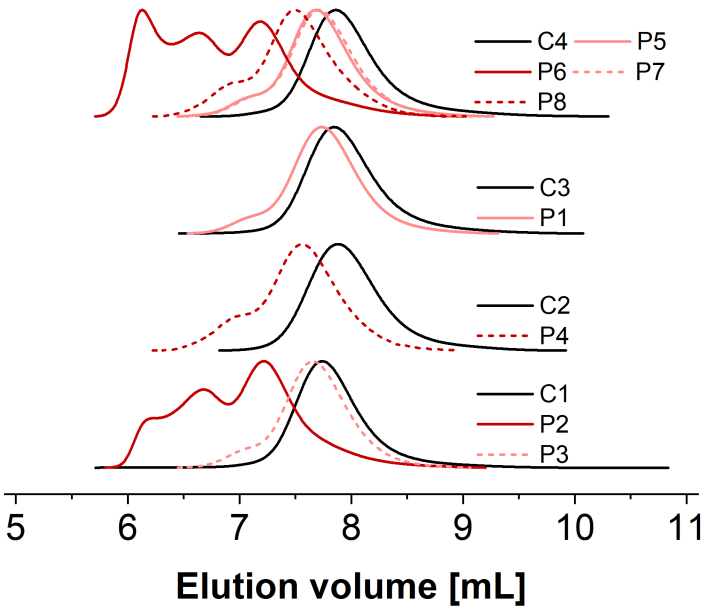


Figure S1: SEC elugrams ($CHCl_3$, 2-PrOH, NEt_3, RI detection) of the hydrophobic PMMA **C1** – **C3** and P(MMA-*stat*-DMAEMA) **C4** (black solid lines) and the corresponding block copolymers with $PEGMA_{360}$ (higher DP: red solid lines, low DP light pink solid lines) and $PEGMA_{500}$ (higher DP: dark red stacked lines, low DP light pink stacked lines).

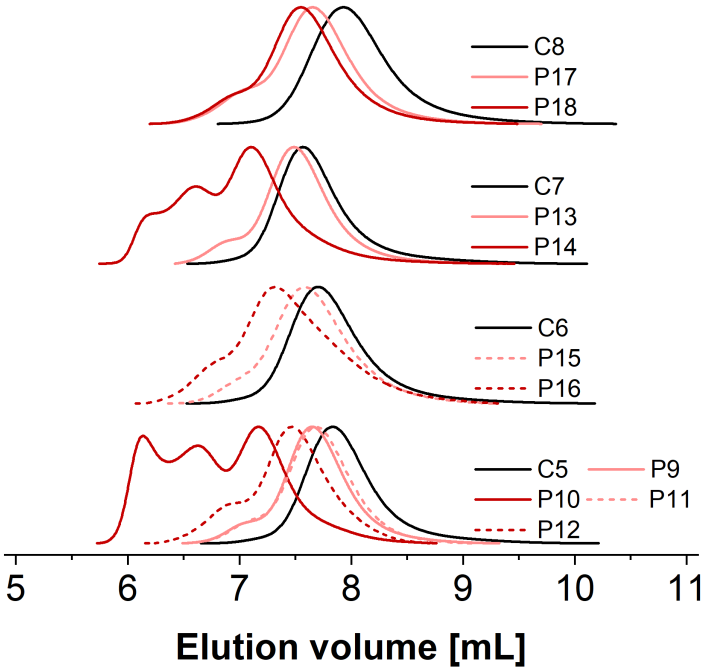


Figure S2: SEC elugrams ($CHCl_3$, 2-PrOH, NEt_3, RI detection) of the hydrophobic P(MMA-*stat*-DMAEMA) **C5** – **C8** (black solid lines) and the corresponding block copolymers with $PEGMA_{360}$ (higher DP: red solid lines, low DP light pink solid lines) and $PEGMA_{500}$ (higher DP: dark red stacked lines, low DP light pink stacked lines).

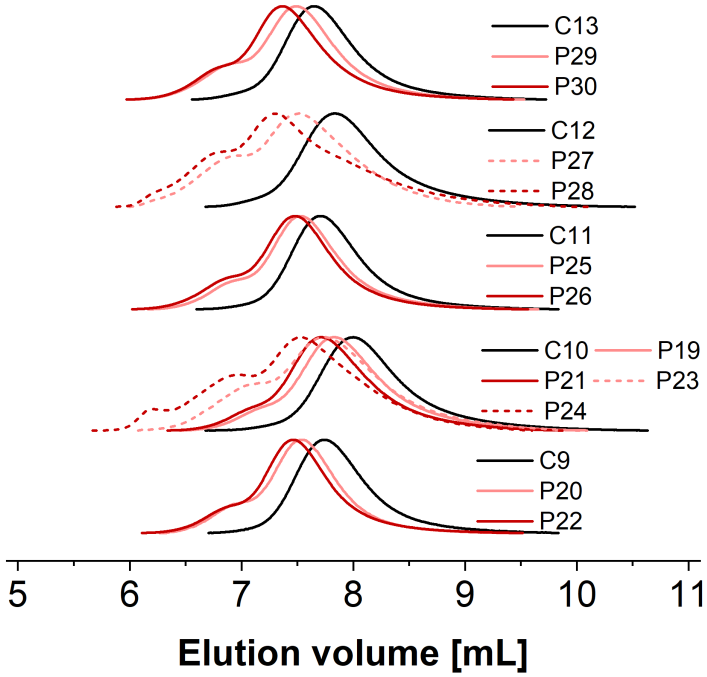


Figure S3: SEC elugrams ($CHCl_3$, 2-PrOH, NEt_3, RI detection) of the hydrophobic P(MMA-*stat*-DMAEMA) **C9** - **C13** (black solid lines) and the corresponding block copolymers with $PEGMA_{360}$ (higher DP: red solid lines, low DP light pink solid lines) and $PEGMA_{500}$ (higher DP: dark red stacked lines, low DP light pink stacked lines).

*UV-absorption of **Pi*** compared to **Pi***

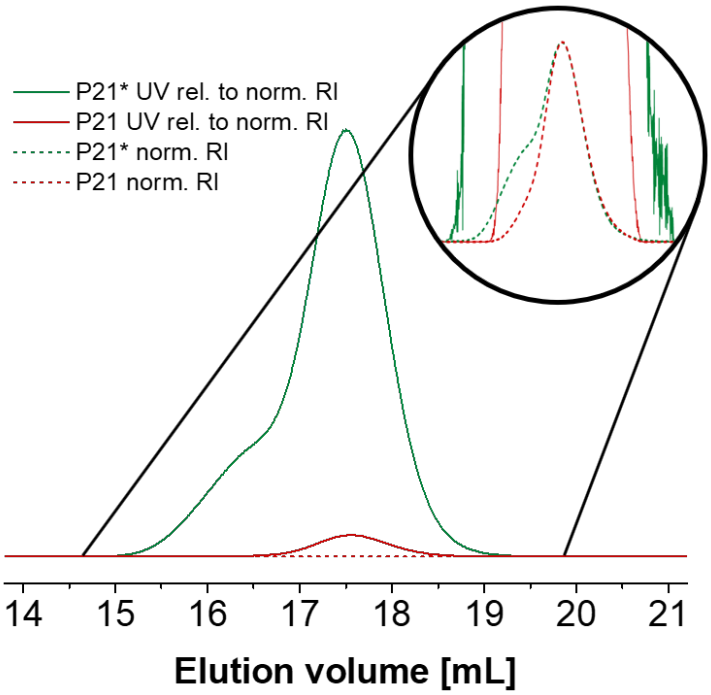
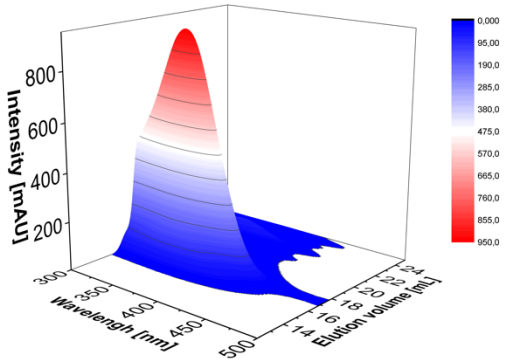


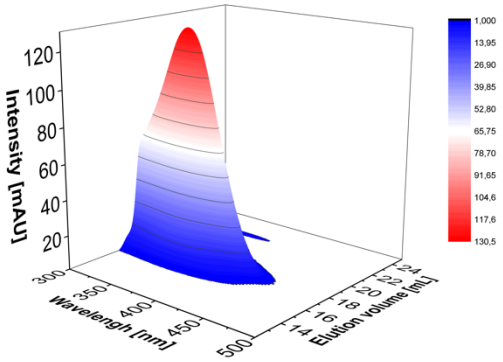
Figure S4: Overlay of SEC elugrams (DMAc, 0.21wt% LiCl) of **P21** (red) and **P21*** (green) with RI detection (stacked lines) and UV detection (solid lines) at λ = 360 nm. Elugrams are normalized according to the maximum RI signal.

*Collection of 3D SEC elugrams of RA-functionalized block copolymers **Pi****

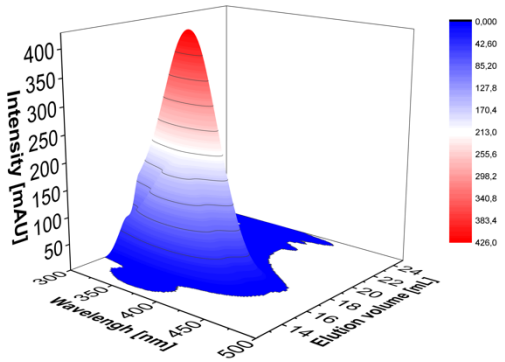
P1*



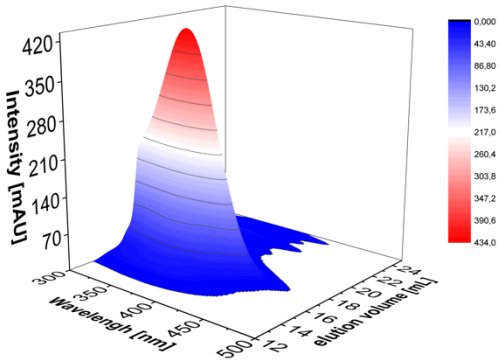
P3*



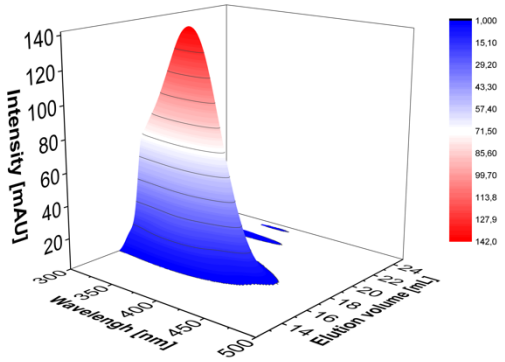
P4*



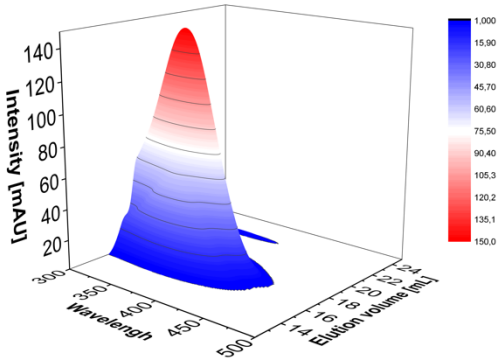
P5*



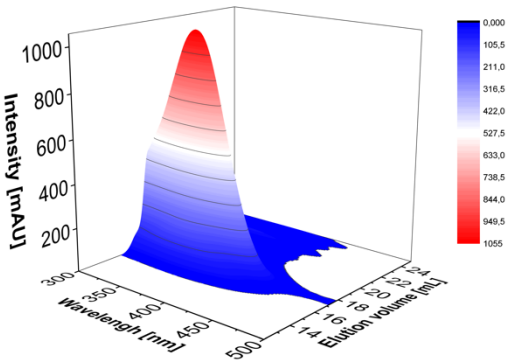
P7*



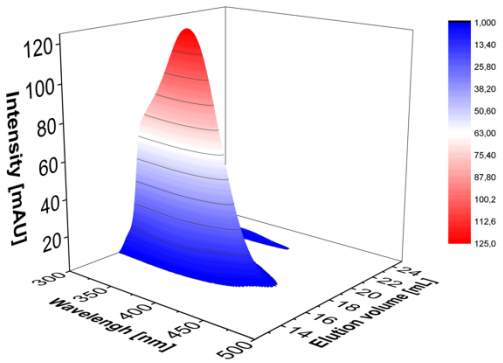
P8*



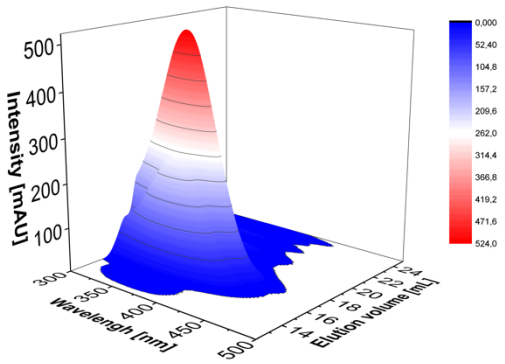
P9*



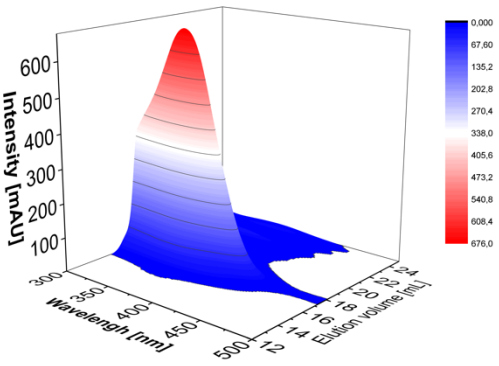
P11*



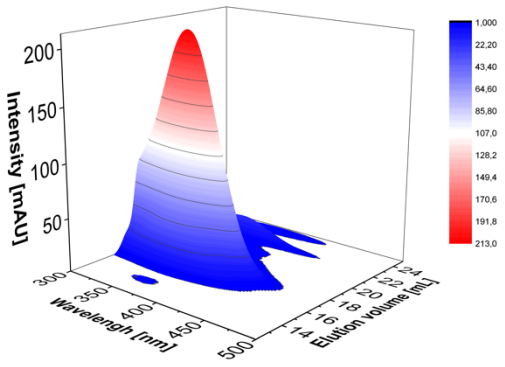
P12*



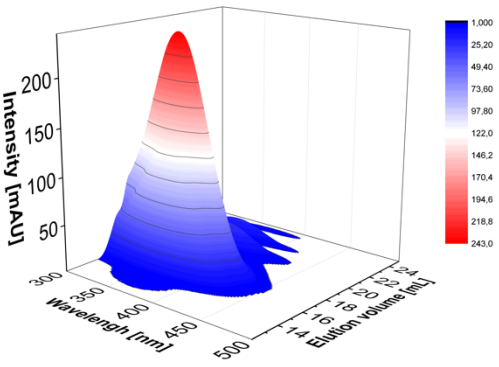
P13*



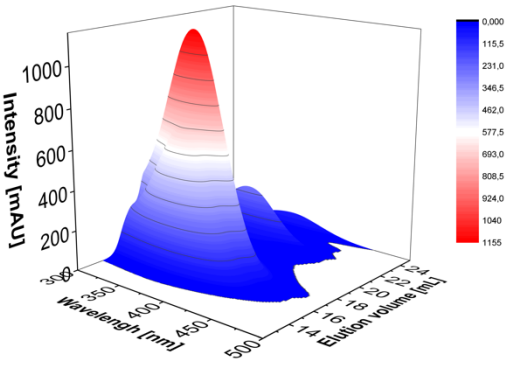
P15*



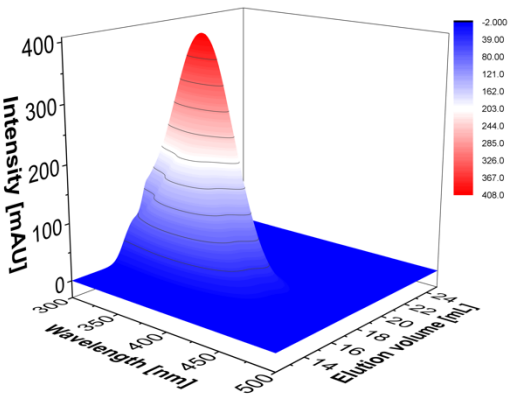
P16*



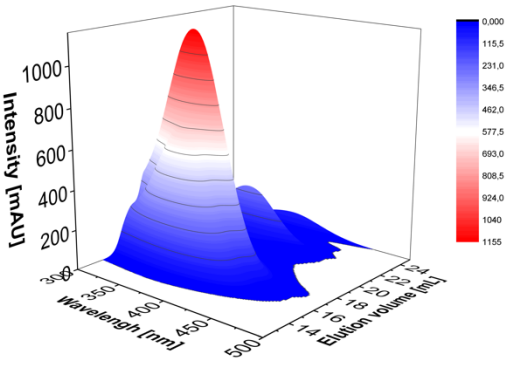
P17*



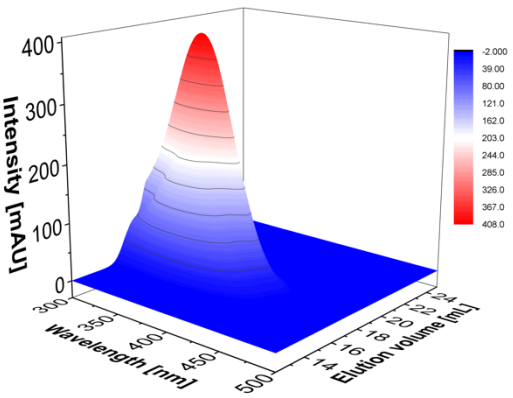
P19*



P20*



P21*



P23*

P24*

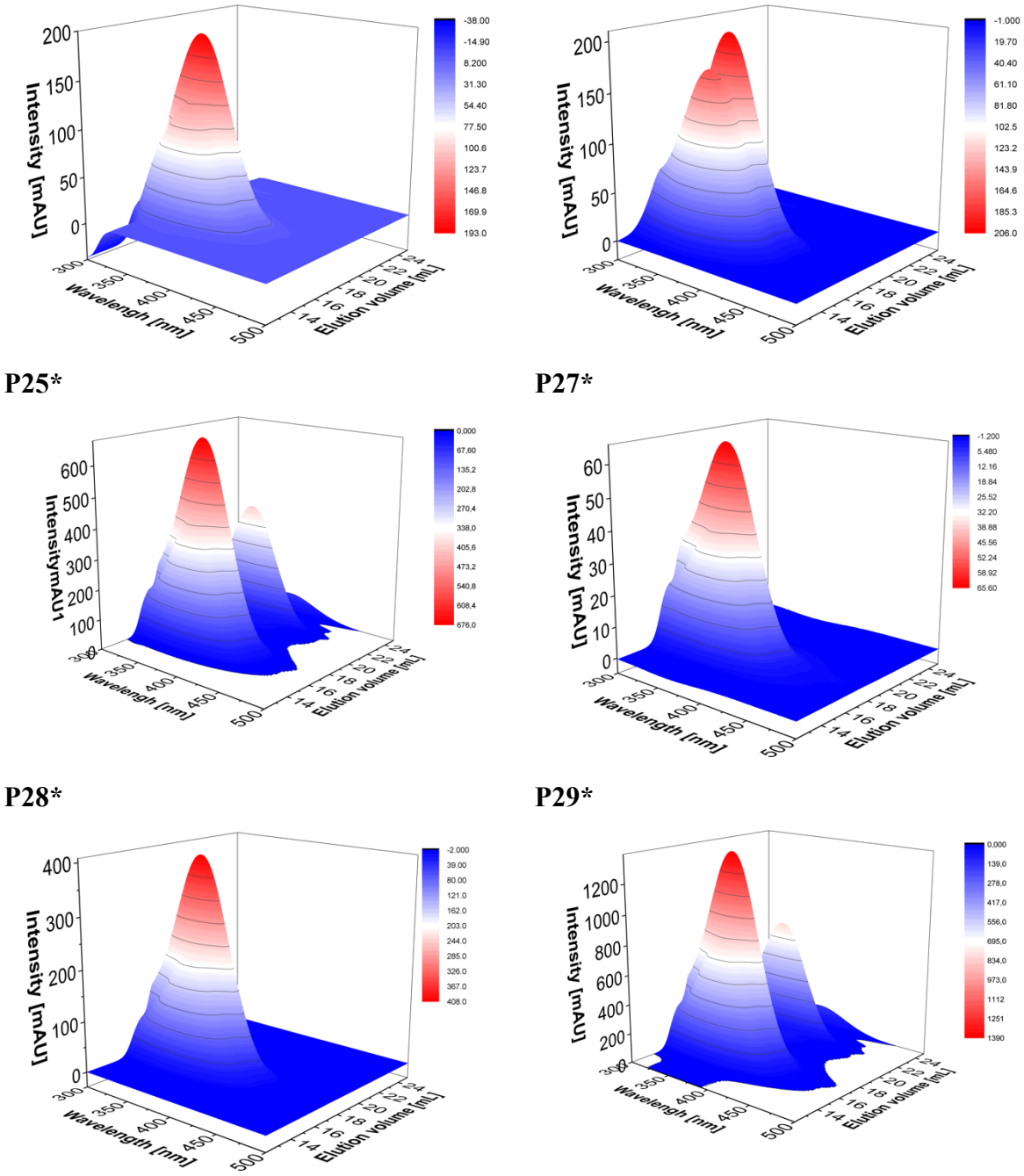


Figure S5: 3D SEC elugrams (DMAc, 0.21wt% LiCl). Absorbance detection λ = 300 to 500 nm.

Calculation of the degree of substitution of retinoic acid

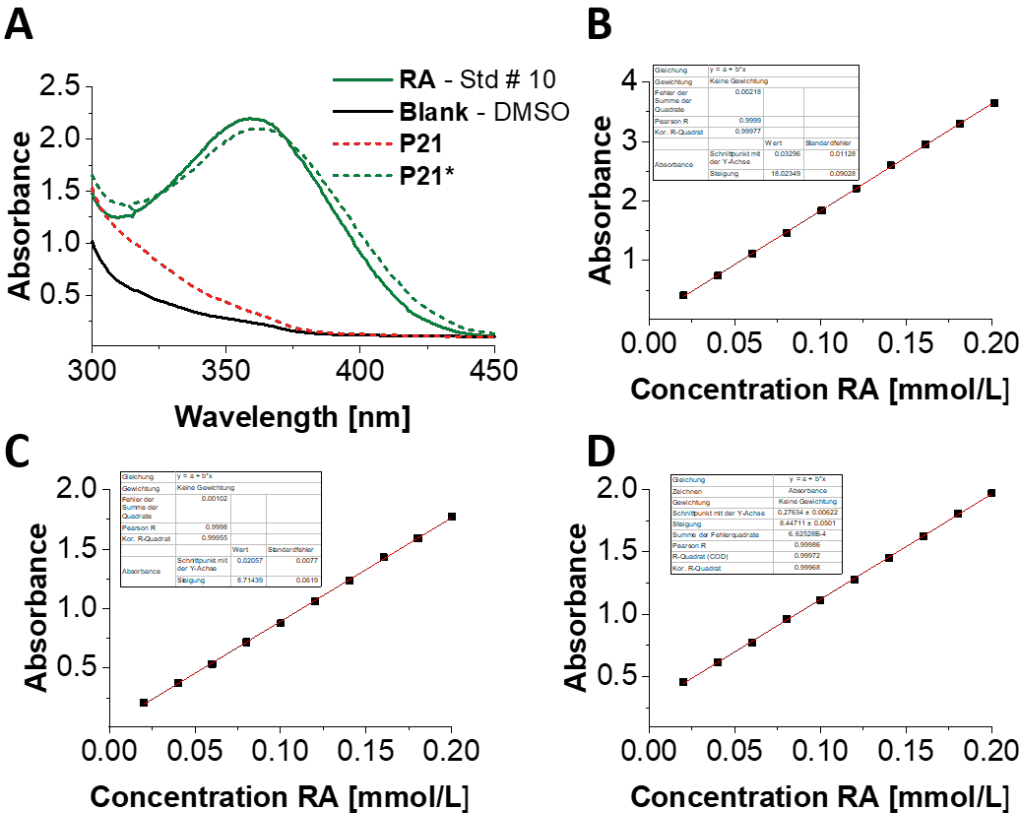


Figure S6: A) Plot absorbance as a function of the wavelength of representatives of RA – STD, blank, **Pi** and **Pi***. B) Plot linear regression calibration 1. C) Plot linear regression calibration 2. D) Plot linear regression calibration 3.

Table S6: Data of absorbance measurements of RA – STDs #1 – 10 and blank at λ = 360 nm for calibration 1 - 3.

RA Std		Absorbance								
		Calibration 1			Calibration 2			Calibration 3		
#	Concentration mmol L^{-1}	1	2	3	1	2	3	1	2	3
10	0.200	3.660	3.661	3.632	1.775	1.772	1.772	1.960	1.978	1.974
9	0.180	3.311	3.305	3.290	1.593	1.591	1.590	1.806	1.771	1.842
8	0.160	2.953	2.957	2.950	1.434	1.433	1.433	1.610	1.622	1.647
7	0.140	2.601	2.597	2.600	1.236	1.235	1.234	1.427	1.462	1.469
6	0.120	2.210	2.210	2.214	1.063	1.062	1.061	1.289	1.268	1.281
5	0.100	1.839	1.839	1.839	0.877	0.876	0.876	1.108	1.110	1.124
4	0.080	1.465	1.464	1.464	0.719	0.718	0.718	0.951	0.974	0.959
3	0.060	1.124	1.122	1.120	0.535	0.534	0.534	0.766	0.768	0.788
2	0.040	0.747	0.746	0.746	0.376	0.377	0.377	0.617	0.604	0.616
1	0.020	0.416	0.415	0.415	0.209	0.209	0.209	0.442	0.440	0.491
(Blank)	0	0.044	0.044	0.043	0.042	0.041	0.041	0.281	0.282	0.249

Table S7: Data of absorbance measurements of **P1 – P30** and **P1* – P30*** at λ = 360 nm, used calibrations, and dilution factors f_{dil}.

Sample	Calibration	Absorbance			f_{dil}	Sample	Calibration	Absorbance			f_{dil}	Sample	Calibration	Absorbance			f_{dil}
		1	2	3				1	2	3				1	2	3	
P1	1	0.084	0.088	0.088	10	**P11**	1	0.095	0.094	0.093	10	**P21**	3	0.349	0.364	0.362	1
P1*	1	0.737	0.741	0.738	10	**P11***	1	0.640	0.639	0.639	10	**P21***	3	1.602	1.534	1.582	1
P2						**P12**	1	0.086	0.082	0.083	10	**P22**					
P2*						**P12***	1	0.853	0.852	0.852	10	**P22***					
P3	1	0.089	0.089	0.090	10	**P13**	1	0.067	0.068	0.069	1	**P23**	3	0.315	0.317	0.352	1
P3*	1	0.614	0.613	0.613	10	**P13***	1	0.412	0.412	0.411	1	**P23***	3	1.276	1.318	1.292	1
P4	1	0.078	0.079	0.079	10	**P14**						**P24**	3	0.319	0.316	0.325	1
P4*	1	1.051	1.049	1.048	10	**P14***						**P24***	3	0.943	1.005	0.976	1
P5	1	0.087	0.088	0.089	10	**P15**	1	0.073	0.074	0.074	10	**P25**	2	0.124	0.124	0.125	1
P5*	1	1.326	1.325	1.324	10	**P15***	1	0.772	0.766	0.765	10	**P25***		0.784	0.740	0.807	10
P6						**P16**	1	0.075	0.075	0.075	10	**P26**					
P6*						**P16***	1	1.079	1.083	1.081	10	**P26***					
P7	1	0.082	0.082	0.082	10	**P17**	2	0.162	0.159	0.160	1	**P27**	3	0.301	0.313	0.313	1
P7*	1	0.981	0.981	0.981	10	**P17***	2	0.551	0.539	0.650	10	**P27***	3	0.649	0.601	0.663	1
P8	1	0.071	0.072	0.072	10	**P18**						**P28**	3	0.306	0.306	0.337	1
P8*	1	0.843	0.843	0.843	10	**P18***						**P28***	3	1.334	1.487	1.454	1
P9	1	0.080	0.082	0.085	10	**P19**	3	0.368	0.345	0.366	1	**P29**	2	0.112	0.112	0.112	1
P9*	1	0.844	0.843	0.842	10	**P19***	3	0.917	0.898	0.919	1	**P29***	2	0.998	0.991	0.999	2
P10						**P20**	2	0.170	0.169	0.169	1	**P30**					
P10*						**P20***	2	0.563	0.451	0.499	10	**P30***					

Calculation of DF_{RA}

$$DF_{RA} = \frac{n_{real}}{n_{100\%}} \cdot 100\% = \frac{n_{real}}{\frac{\left(\frac{DP_{PEGMA} \cdot M_{n,PEGMA}}{M_{n,theo,Pi}} \cdot m_{Pi^*}\right)}{M_{n,PEGMA}}} \cdot 100\% = \frac{n_{real} \cdot M_{n,theo,Pi}}{DP_{PEGMA} \cdot m_{Pi^*}} \cdot 100\%$$

$$= \frac{(n_{Pi^*} - n_{Pi}) \cdot M_{n,theo.Pi}}{DP_{PEGMA} \cdot m_{Pi^*}} \cdot 100\% = \frac{(A_{Pi^*} - A_{Pi}) \cdot V \cdot M_{n,theo,Pi}}{DP_{PEGMA} \cdot m_{Pi^*} \cdot a_{LR} \cdot f_{dil}} \cdot 100\%$$

$$DF_{RA} = \frac{n_{real}}{n_{100\%}} \cdot 100\% = \frac{(A_{Pi^*} - A_{Pi} - A_{DMSO}) \cdot V_{sample} \cdot M_{n,theo,Pi}}{DP_{PEGMA} \cdot m_{Pi^*} \cdot a_{LR} \cdot f_{dil}} \cdot 100\% \qquad \text{(S6)}$$

n_{real}	Real quantity of RA
$n_{100\%}$	Molar amount of RA if PEGMA completely functionalized
DP_{PEGMA}	Degree of polymerization of PEGMA
A_{Pi^*}	Absorbance Pi* sample at $\lambda = 360\ nm$
A_{Pi}	Absorbance Pi sample at $\lambda = 360\ nm$
A_{DMSO}	Absorbance DMSO at $\lambda = 360\ nm$
V_{sample}	Volume of the Pi* sample
$M_{n,theo,Pi}$	Molar mass of Pi
m_{Pi^*}	Mass of Pi*
a_{LR}	Rise calculated from linear regression
f_{dil}	Dilution factor of Pi* sample
DF_{RA}	Degree of functionalization of RA

Raman spectroscopic investigation of representatives of the library

FT-Raman spectra were recorded on a Bruker MultiSpec spectrometer using 1 W of excitation power at 1064 nm. Spectra were recorded up to 4000 cm^{-1} with a spectral resolution of 4 cm^{-1} and averaged over 256 scans. Background subtraction was performed in the Bruker Opus 6.5 software package using the built-in "Polynomial" function and an appropriate amount of iterations (4-6, with the only exception being the sample **C3** (23)).

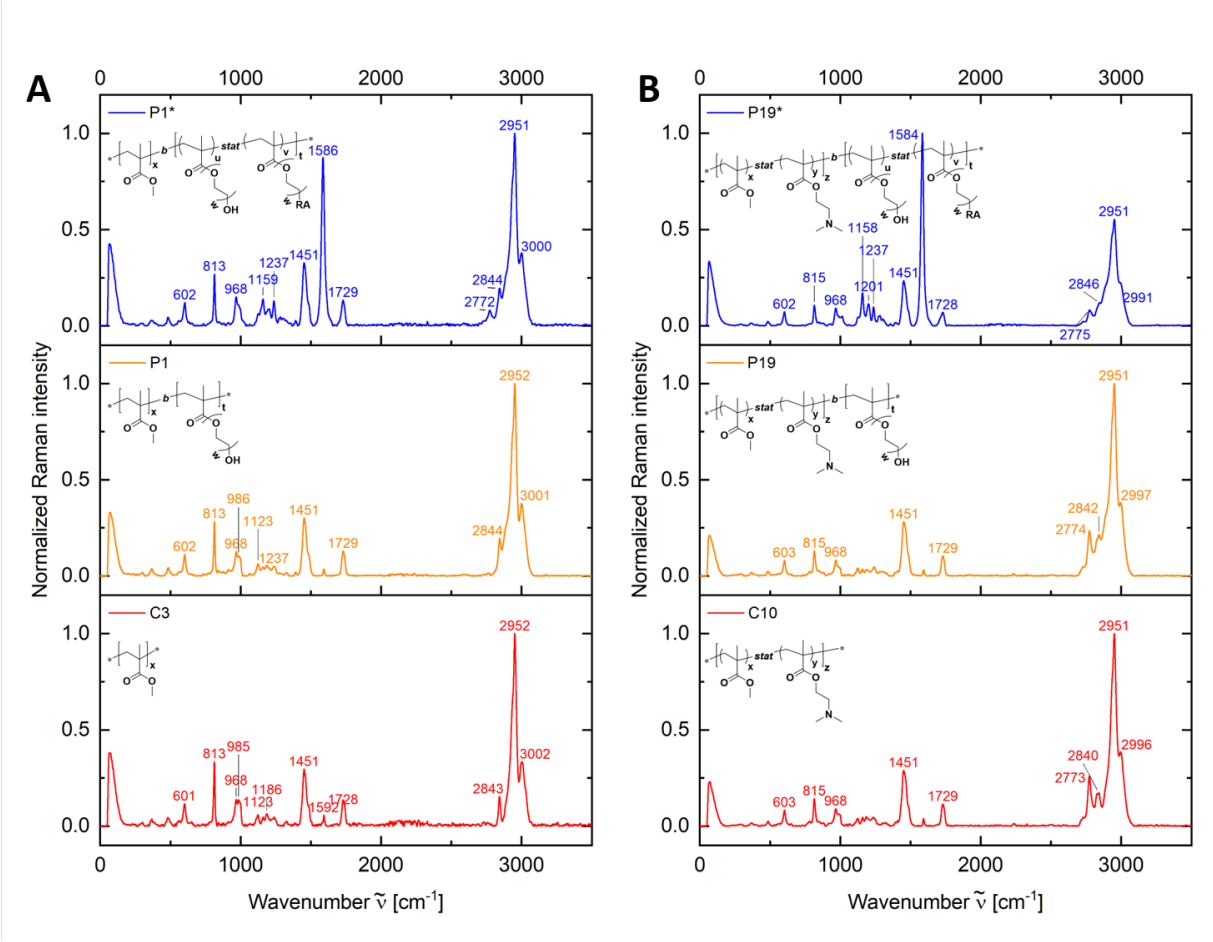


Figure S7: Background corrected and normalized FT-Raman spectra of A) **C3**, **P1**, **P1*** (0% DMAEMA) and B) **C10**, **P19**, **P19*** (30% DMAEMA) as bulk material. The characteristic retinyl resonances at 1586 cm^{-1} (A, **P1***) and 1584 cm^{-1} (B, **P19***) are dominant features in the final products' spectra, despite DS values of 11% (**P1***) and 9% (**P19***), highlighting the particularly large Raman cross section.

Schematic illustration of SERS measurement

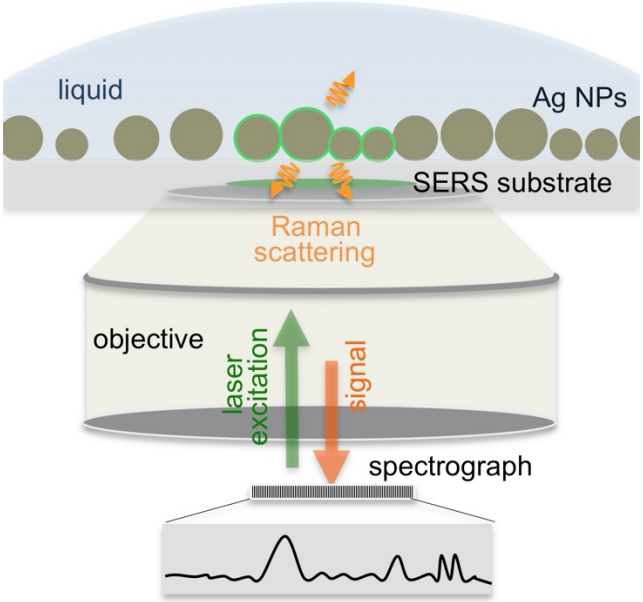


Figure S8: Scheme of the SERS measurements with Ag nanoparticle decorated glass substrates in liquid environment.

Peak assignment of the SERS measurements

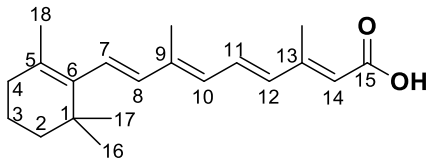


Figure S9: Numbering of retinoic acid atoms for peak assignment.

The intense peak at 1569 cm^{-1} is usually assigned to a degenerated band comprising the C11=C12 stretching vibration and C9=C10 stretching vibration and incorporate significant contributions of the in-plane CCH rocking modes. The two C-C stretching modes found in the Raman spectrum of pure retinoic acid have been previously specifically assigned to C10-C11 stretching mode (ν_{cc} = 1151 cm^{-1}) and to the C8-C9 stretching mode ($\nu_{c\text{-}c}$ = 1198 cm^{-1}) in all-trans-retinal.[1] The C14-C15 stretch has been localized in all-trans-retinal at 1111 cm^{-1}, however, modifications at C15 have been shown to influence the position of this band. As such, the C14-C15 stretching mode has been shown to be largely localized at approximately 1202 cm^{-1} for the all-trans-retinal chromophores in bacteriorhodopsin and at 1240 cm^{-1} in PSB-all-trans retinal.[2] Therefore, alternatively to the assignment of the 1226 cm^{-1} band found in the NP comprising retinoyl moieties to C-O-C stretching mode of the PEGMA units, this Raman band can be also assigned to a retinol specific signature, induced by the covalent binding of the retinoic acid to the PEGMA unit yielding a retinoyl moiety. The formed ester bond results in a carbonyl band

at 1700 cm^{-1} and leads to a modification of the π -electron delocalization, which affects the ground state order of the C14-C15 bond and, thus, might induce a shift of the C-C stretching mode to 1226 cm^{-1}.

Table S8: Peak assignment for the SERS measurements shown in **Figure 6A**.

Raman mode[a)]	Retinoic acid cm^{-1}	P20* cm^{-1}	P20 cm^{-1}
C=C stretching modes	1572	1559	-
CH rocking mode	1267	1269	-
C-C stretching	1191	1192	-
C-C stretching	1151	1158	-
CH in plane rocking mode	1006	1000	-
H out of plane wagging mode	962	969	-
C-C stretching	-	1114	-
C-C stretching	-	1226	-
C=C stretching	-	-	1584
Intensity ratio			
I_{1569}/I_{1151}	6	6.0	-
I_{1669}/I_{1226}	-	6.15	-

a) Peak assignment according to Ref. [1].

AUC investigations

P24 was investigated on its own and further in the presence of RBP in solution at a rotor speed of 7,500 rpm for 18 h, in the same AUC run as **P24*** with instrumental parameters as explained in the experimental section of the manuscript. After sedimentation velocity experiments of the NPs at lower rotor speeds as described, the rotor speed was further increased to 42,000 rpm and hold constant for 24 h, in order to observe the sedimentation of the proteins present in the samples. As a control, the potential specific interaction of NPs with RBP was further studied in comparison with the interaction of NPs with BSA. For this purpose, a stock solution of BSA at a concentration of c = 2 mg/mL was prepared in 0.01 M PBS buffer solution and the final concentration of BSA in the sample was adjusted to c ≈ 0.5 mg/mL.

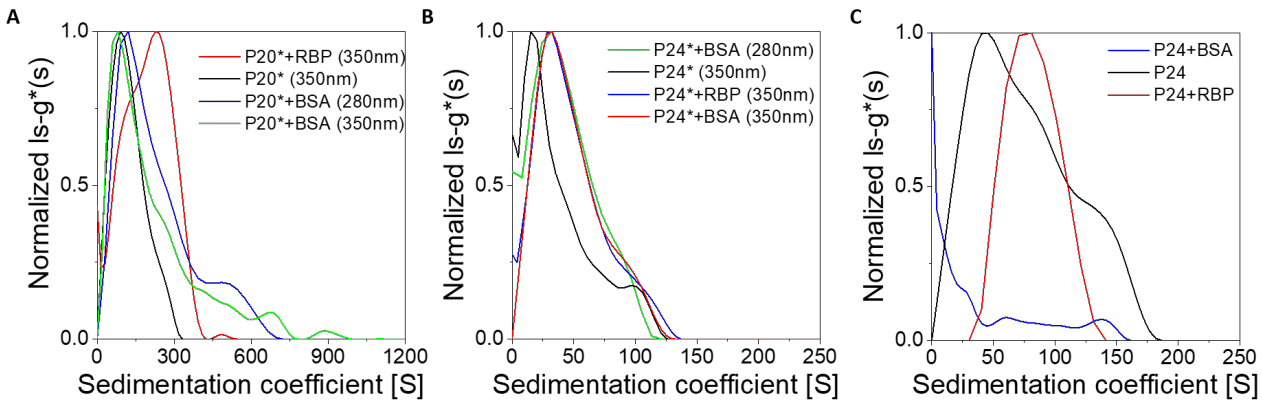


Figure S10: Normalized differential distribution of sedimentation coefficients, ls-g*(s), A) from using a wavelength of $\lambda = 350$ nm or $\lambda = 280$ nm in terms of OD of **P20*** ($c = 0.55$ mg mL^{-1}) and also with BSA at the following concentrations: $c_{NP} = 0.19$ mg mL^{-1}, $c_{BSA} = 0.51$ mg mL^{-1} or RBP ($c_{NP} = 0.12$ mg mL^{-1}, $c_{RBP} = 0.21$ mg mL^{-1}). B) The same as in A) for **P24*** at the following concentrations: $c = 0.54$ mg mL^{-1}, with BSA ($c_{NP} = 0.50$ mg mL^{-1}, $c_{BSA} = 0.49$ mg mL^{-1}) and with RBP ($c_{NP} = 0.5$ mg mL^{-1}, $c_{RBP} = 0.29$ mg mL^{-1}). C) Normalized differential distribution of sedimentation coefficients of **P24** at $c = 0.54$ mg mL^{-1} and additionally with BSA at the following concentrations: $c_{NP} = 0.5$ mg mL^{-1}, $c_{BSA} = 0.5$ mg mL^{-1} or RBP ($c_{NP} = 0.5$ mg mL^{-1}, $c_{RBP} = 0.21$ mg mL^{-1}) in solution, all monitored at a wavelength of $\lambda = 280$ nm.

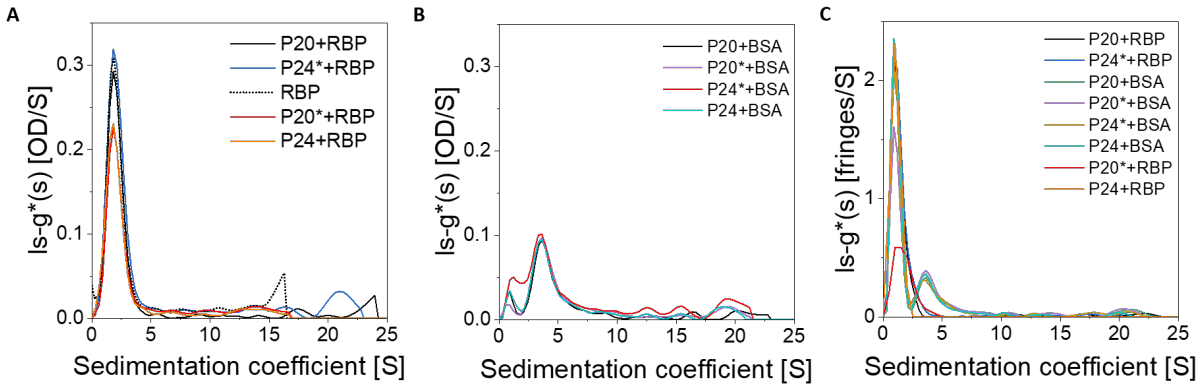


Figure S11: Differential distribution of sedimentation coefficients, ls-g*(s), after acceleration of the rotor speed to 42,000 rpm and data acquisition at a wavelength of $\lambda = 280$ nm in terms of OD of A) RBP and all NP samples containing RBP in solution, and B) all NP samples containing additionally BSA in solution. C) Differential distribution of sedimentation coefficients, ls-g*(s), recorded with the interference detection system in terms of interference fringes of all NP samples containing either RBP or BSA in solution.

Stability tests of representatives at different pH-values

Table S9: pH responsiveness of the tested nanoparticles.

Sample	pH 4.5 Acetate buffer	pH 5.6 Acetate buffer	pH 6.0 Citrate buffer	pH 7.4 Phosphate buffer
P4	dissolved	dissolved	dissolved	stable
P19	stable	stable	stable	stable
P29	dissolved	dissolved	dissolved	dissolved

Gel electrophoresis

Agarose powder was suspended in TAE-buffer (1.5%) and heated for complete dissolution. Ethidium bromide (40 µL of 1 mg mL^{-1}) were added to 40 mL cooled but not gelled agarose solution and poured into an assembled gel tray with an appropriate comb. 5 µL ladder was used for each run. The concentration of the used working solutions and the mixture of the samples are shown in detail in **Tables S11-13**. Agarose gel electrophoresis was run at room temperature at 100 V, 2 mA for 30 to 60 min in 1x TAE-buffer containing 40 µL of a 1 mg mL^{-1} ethidium bromide solution.

Next to the ability to encapsulate or adsorb genetic material, the nanoparticles stability and ability to protect the loaded tRNA was investigated. RNase A, which destroys free tRNA, was used for this study. Heparin is able to release genetic material after complexation/encapsulation but also protects it against RNase A. After a 60 min treatment of the particles with RNase A, heparin was added to release the tRNA and to protect it against still active RNases at the same time. The particles protected the tRNA and were still able to release it while the free tRNA was successfully degraded (**Tables S11-S13**, **Figure S12**).

Table S10: Solutions for gel electrophoresis.

Working solution	concentration
tRNA	20 µg mL^{-1}
nanoparticles	2 mg mL^{-1}
Heparin	15.000 U mL^{-1}
RNase	200 µg L^{-1}

Table S11: Tested samples in gel electrophoresis.

Abbreviation	Sample
t	tRNA
t_R	RNase treated tRNA
A	**P19**, tRNA adsorption
B	**P19**, tRNA encapsulated
C	**P4**, tRNA adsorption
D	**P4**, tRNA encapsulated
E	**P29**, tRNA adsorption
F	**P29**, tRNA encapsulated

Table S12: Mixing ratios for gel electrophoresis.

Test type	$V_{Sample/tRNA}$	$V_{Heparin}$	V_{RNase}	V_{Dye}
	µL	µL	µL	µL
untreated	5.0	-	-	1.0
Heparin detachment	5.0	1.0	-	1.2
RNase stability	5.0	1.0/0*	1.0	1.4/1.2*

*tested tRNA sample

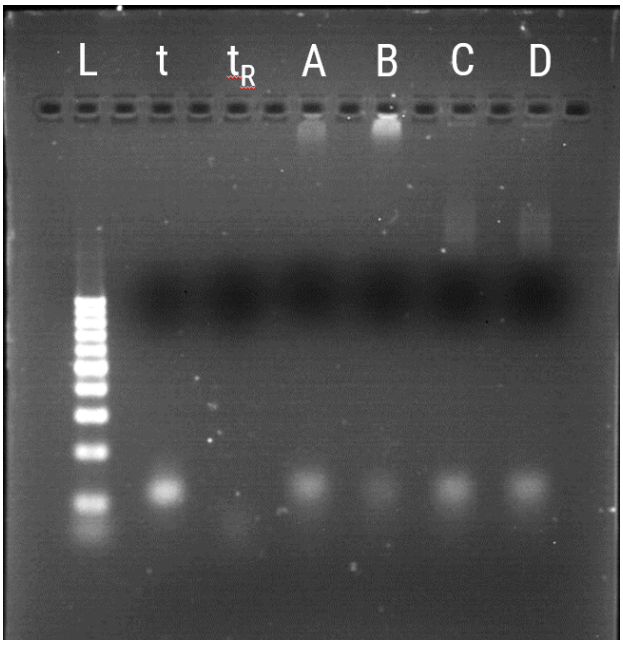


Figure S12: Gel electrophoresis showing RNase stability investigations. For sample codes see **Table S12.**

SDS PAGE

The buffers used for the native PAGE and the composition of the samples are shown in **Table S14**. One hour after mixing, the samples were loaded into precast Mini-PROTEAN 4-15% gels. Electrophoresis was performed in 1× running buffer (5× running buffer: 125 mM Tris base, 0.96 M glycine) at room temperature for 60 min using a constant voltage (200 V).

Table S13: Sample compositions used for native PAGE experiments.

Sample	RBP[a)]	NPs[b)]	Water	Sample buffer
	µL	µL	µL	µL
Reference	2.4	-	1.6	4.0[c)]
Tested NPs	2.0	18.8/37.6[d)]	-	5.2[e)]

a) c = 0.25 mg mL^{-1}. b) c = 1 mg mL^{-1}, for **P20** c = 1 mg mL^{-1}. c) buffer composition: 3.75 mL 0.5 M Tris-HCl (pH = 6.8), 24 mL 50% glycerol, 0.3 mL 1% Bromophenol blue. d) for **P20** 37.6 µL. e) buffer composition: 1.9 mL 0.5 M Tris-HCl (pH = 6.8), 12 mL glycerol, 0.6 mL 1% Bromophenol blue; for **P20** 9.9 µL.

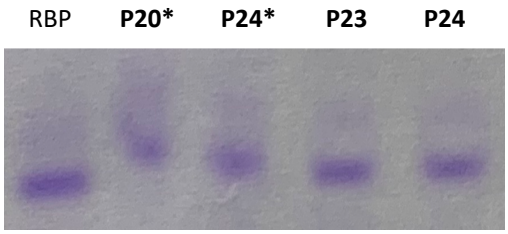


Figure S13: Native-PAGE analysis of Human Urine RBP. RBP preincubated with RA-containing and non-labeled nanoparticle suspensions. The gel bands were stained with Coomassie brilliant blue.

Fluorescence Microscopy

Imaging parameters (common to all measurements):

1.4 NA 63x oil immersion objective (HC PL APO CS2), immersion oil: r = 1.518

Imaging parameters (specific):

Figure 7B-D, **Figure S14-21**: 2048 x 2048 px, 100 scans, pinhole: 1 A.U. (@565 nm), Scan speed: 600 Hz, ex.: 540 nm, det.: 555 to 701 nm, zoom 2, pixel size (x,y): 0.0450714 µm, hybrid detector (photon counting), ca. 0.5 mW ex. power

Figure 8A/B: 2048 x 2048 px, 6 line accumulations, 4 frame averages, pinhole: 1 A.U. (@565 nm), Scan speed: 600 Hz, ex.: 540 nm, det.: 550 to 650 nm in intervals of 5 nm (20 frames), zoom 2, pixel size (x,y): 0.0450714 µm, hybrid detector (gain: 500, time gating: 0.3 ns to 12.0 ns), 16 bit, ca. 1 mW ex. Power

Figure 8C: 792 x 792 px, 28 z-sections spaced 0.125 µm, 10 frame averages, pinhole: 0.5 A.U. (@565 nm), Scan speed: 400 Hz, ex: 540 nm, det: 555 to 701 nm, zoom 6, pixel size (x,y): 0.0388804 µm, hybrid detector (gain: 500), 8 bit (upconverted to 16 by Lightning algorithm), ca. 1 mW ex. Power.

On the following pages, 16 datasets are shown in two different representations, illustrating the different amounts of fluorescence observed in MEF cells after one hour of incubation with 30% DMAEMA (**P22**) NP loaded with Cy3-siRNA (**Figures S14**, **S18**), 40% DMAEMA (**P22**) NP loaded with Cy3-siRNA (**Figures S15**, **S19**), nothing (ctrl, **Figures S16**, **S20**) and Cy3-siRNA (**Figures S17**, **S21**). Four positions per group are shown. The first representation (**Figures S14-S17**) uses the same brightness scaling (256 color values coding for 0 to 255 photons in any given pixel, with pixels with 255 or more photons being depicted as white) to facilitate comparability between treatment groups. The second coloring (**Figures S18-S21**) is optimized to the individual images. The brightness scaling was performed such that the brightest 1% of pixels of any given image was saturated, with the remaining values being mapped linearly to the shown color scheme.

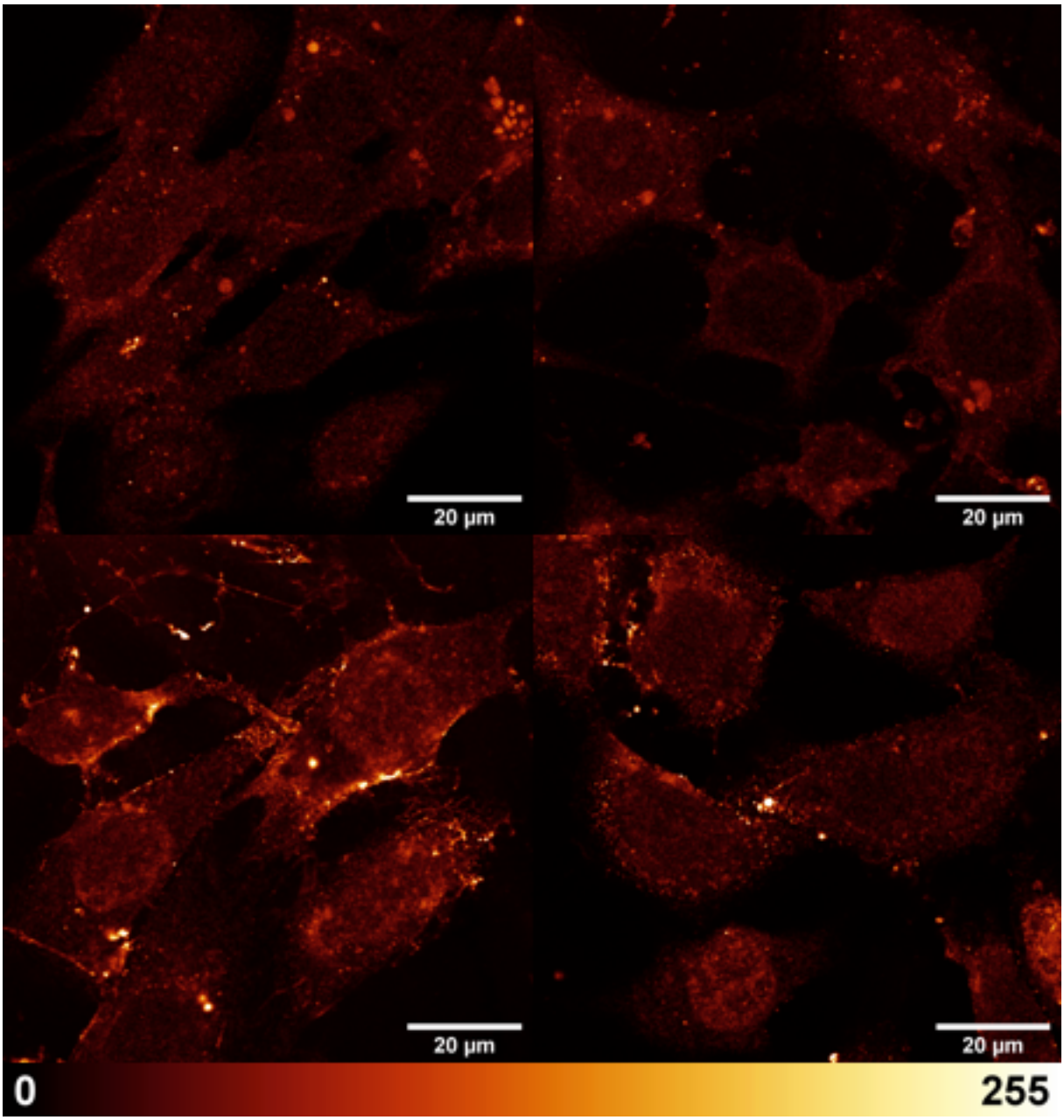


Figure S14: Fixed MEF cells after one hour of incubation with 30% DMAEMA (**P22**) NP loaded with Cy3-siRNA (global color scheme). The bottom left image is equivalent to **Figure 7B** and only given for reference.

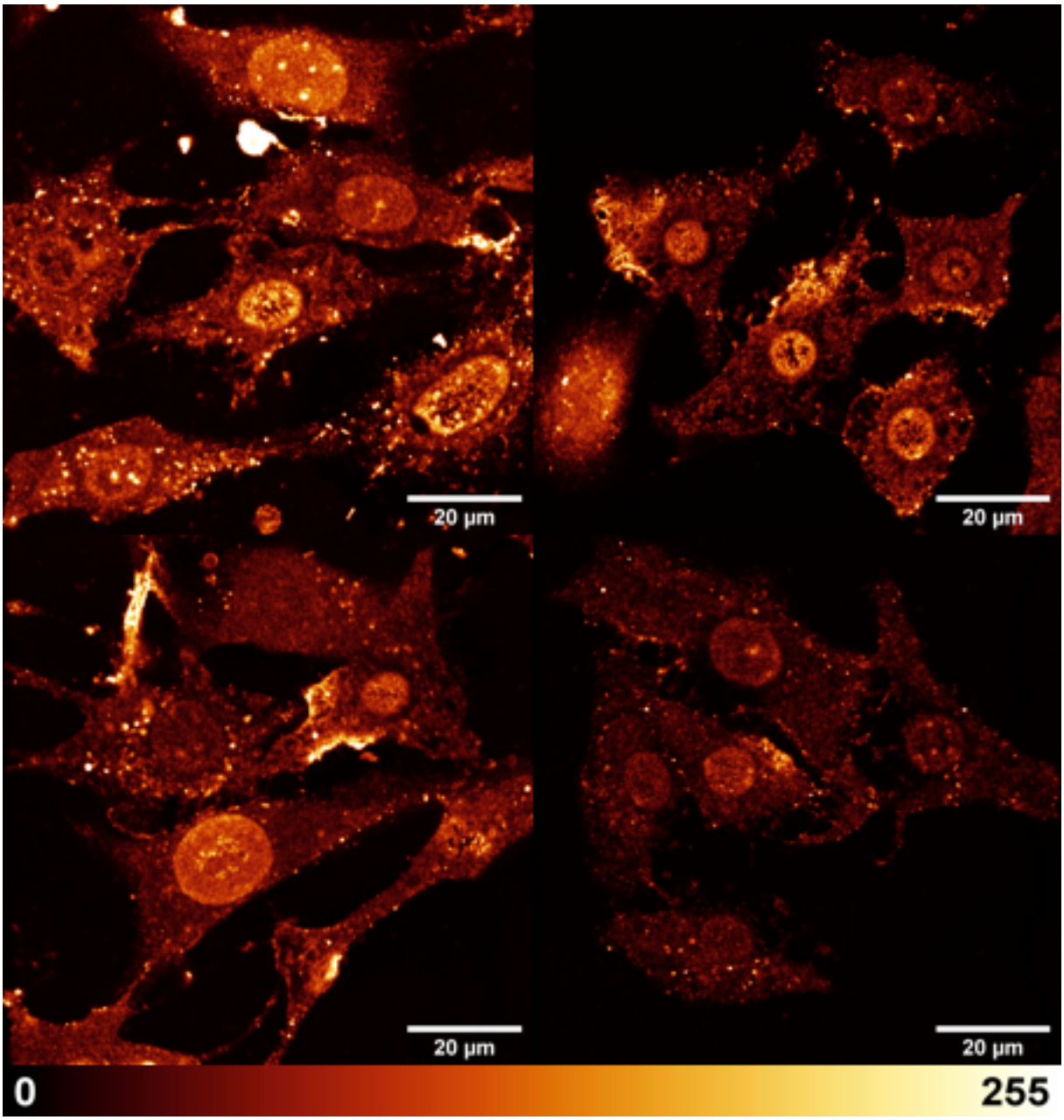


Figure S15: Fixed MEF cells after one hour of incubation with 40% DMAEMA (**P26**) NP loaded with Cy3-siRNA (global color scheme). The top left image is equivalent to **Figure 7C** and only given for reference.

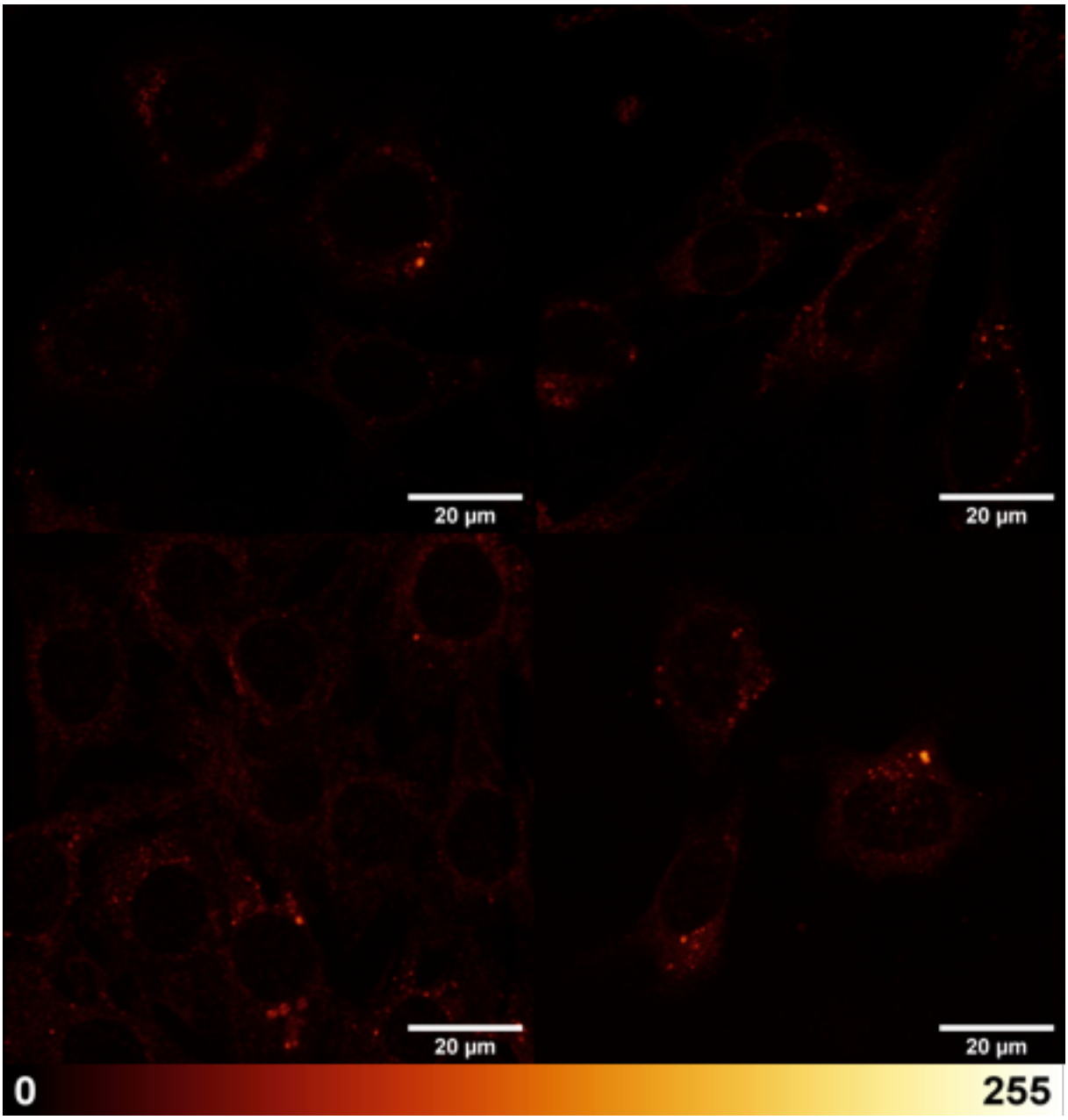


Figure S16: Fixed control MEF cells after one hour (global color scheme). The top left image is equivalent to **Figure 7D** and only given for reference.

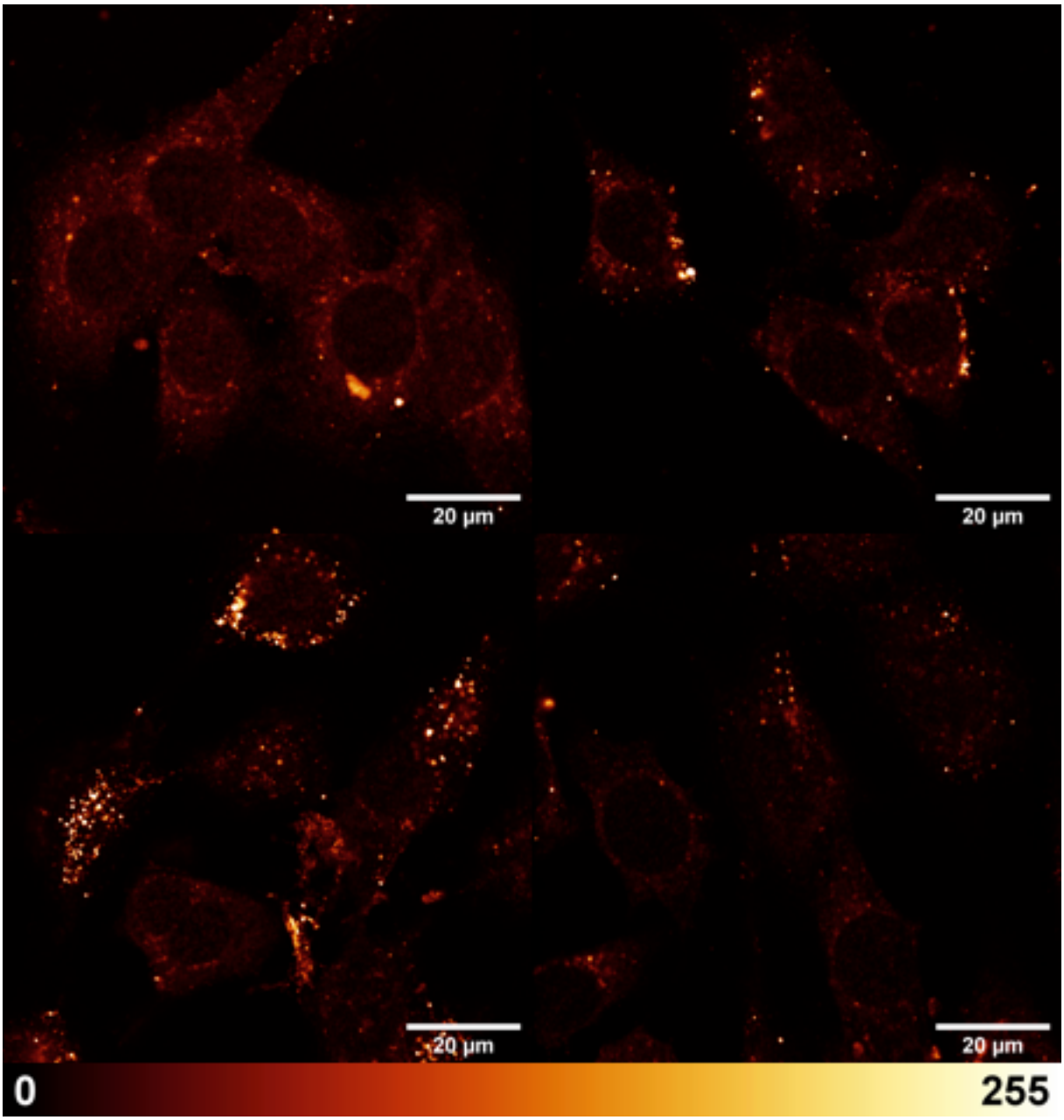


Figure S17: Fixed MEF cells after one hour of incubation with Cy3-siRNA (global color scheme).

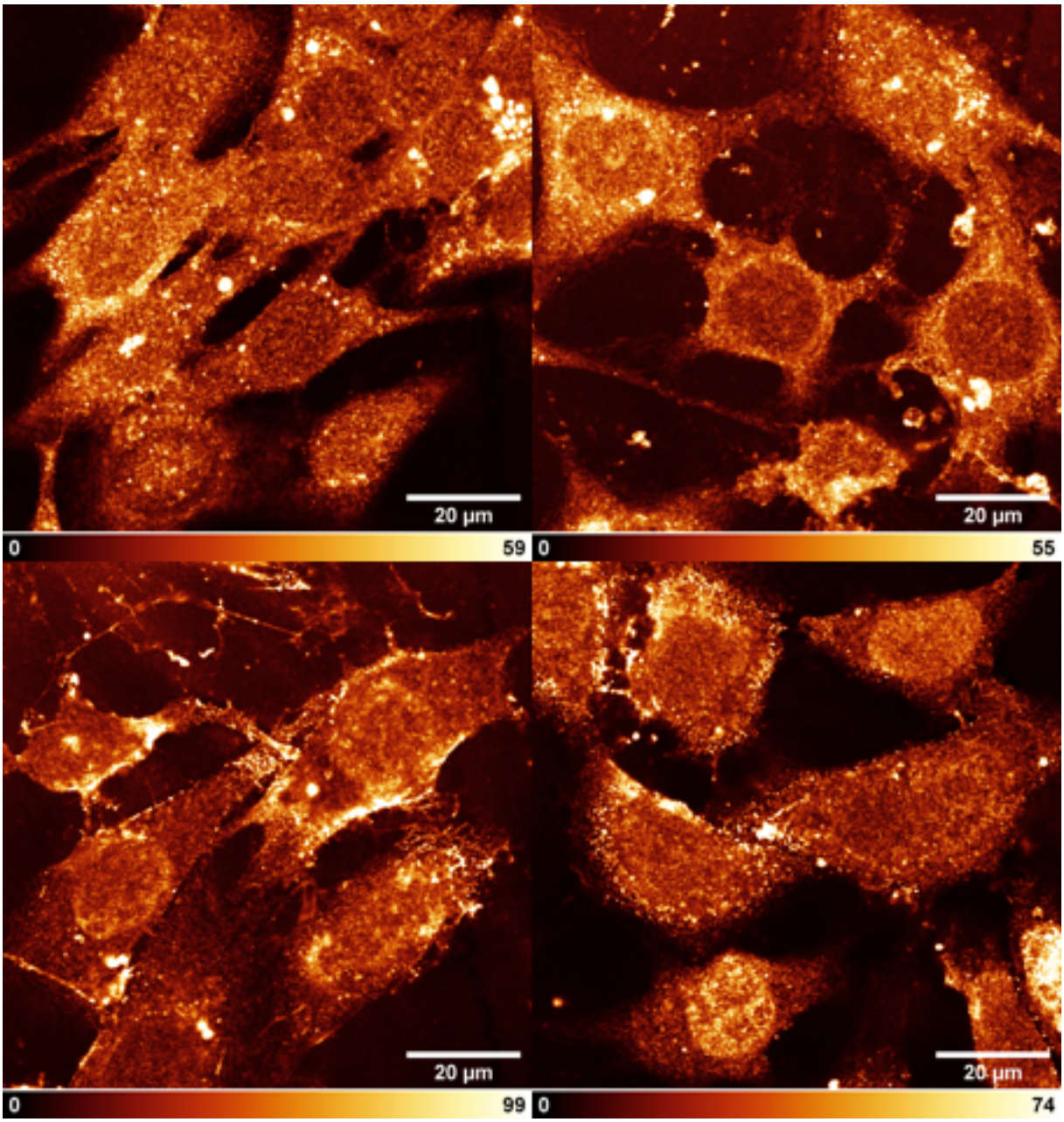


Figure S18: Fixed MEF cells after one hour of incubation with 30% DMAEMA (**P22**) NP loaded with Cy3-siRNA (individual color scheme).

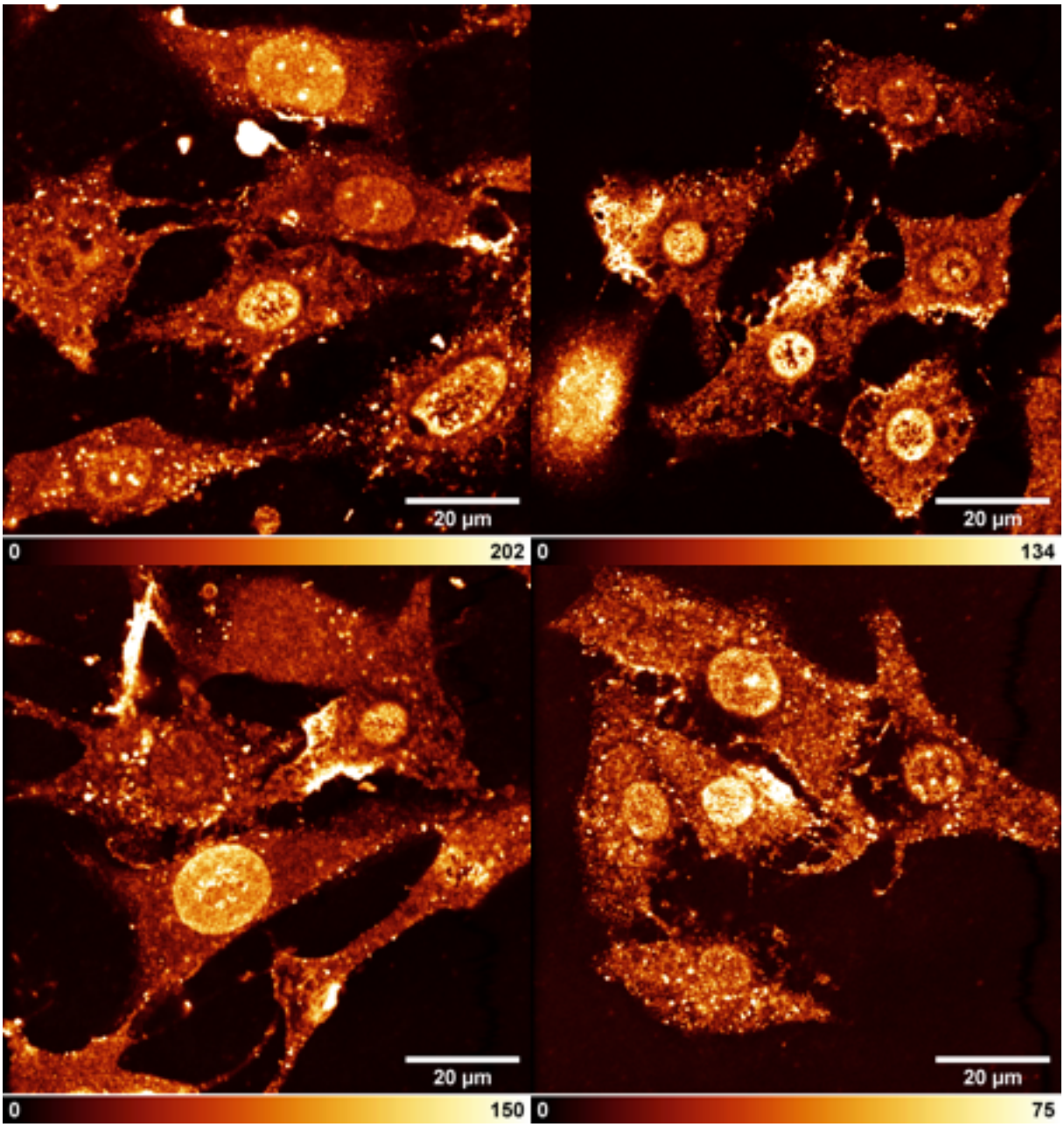


Figure S19: Fixed MEF cells after one hour of incubation with 40% DMAEMA (**P26**) NP loaded with Cy3-siRNA (individual color scheme).

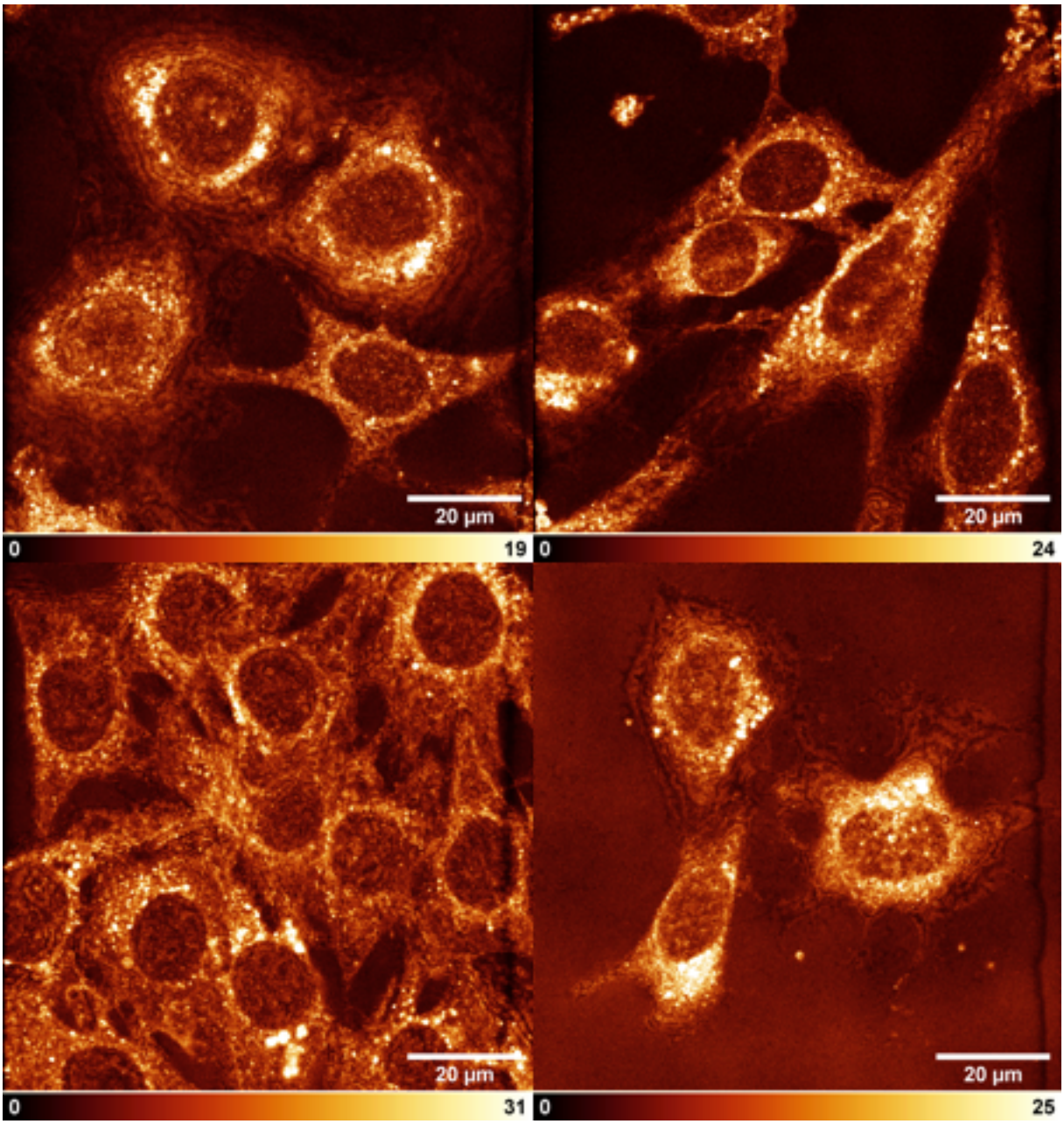


Figure S20: Fixed control MEF cells after one hour (individual color scheme).

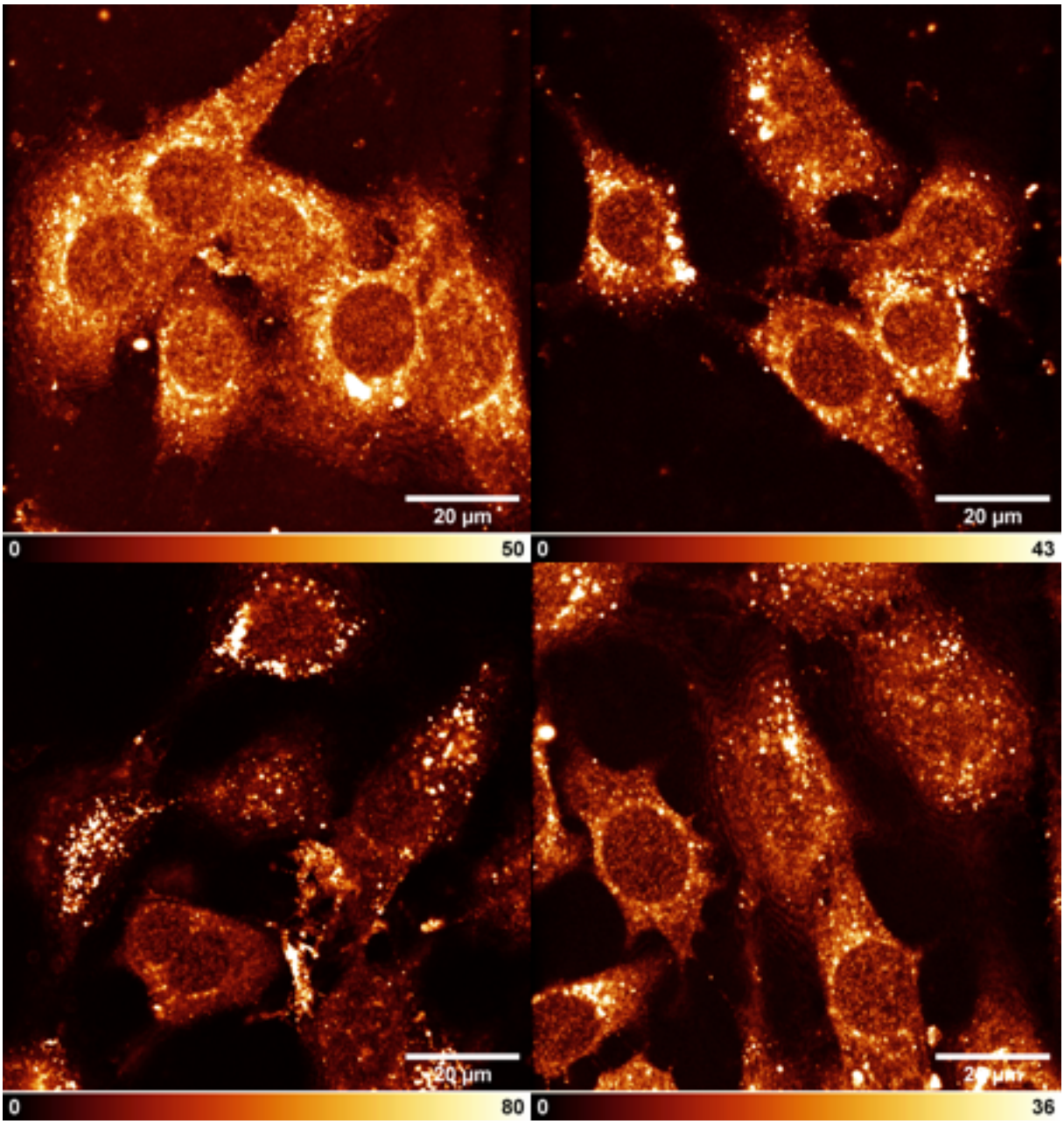


Figure S21: Fixed MEF cells after one hour of incubation with Cy3-siRNA (individual color scheme).

References

1. Curry, B.; Broek, A.; Lugtenburg, J.; Mathies, R., Vibrational analysis of all-trans-retinal. *J. Am. Chem. Soc.* **1982**, 104, 5274-5286.
2. Smith, S. O.; Braiman, M. S.; Myers, A. B.; Pardoen, J. A.; Courtin, J. M. L.; Winkel, C.; Lugtenburg, J.; Mathies, R. A., Vibrational analysis of the all-trans-retinal chromophore in light-adapted bacteriorhodopsin. *J. Am. Chem. Soc.* **1987**, 109, 3108-3125.