Supporting Information

Potential Toxicity of Up-converting Nanoparticles Encapsulated with a Bilayer Formed by Ligand Attraction

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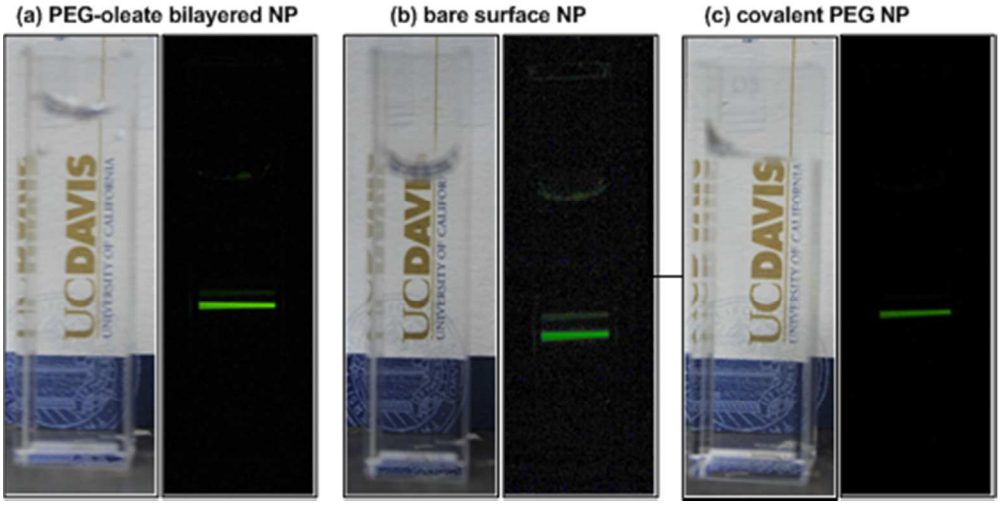


Figure S1. Cuvettes of nanoparticle dispersion in water after different surface modifications (left panel). Green line of NPs emission is seen upon 980 nm excitation (right panel in each case). NPs concentrations in all cuvettes were 1 mg/ml with a laser power 65 W/cm^2.

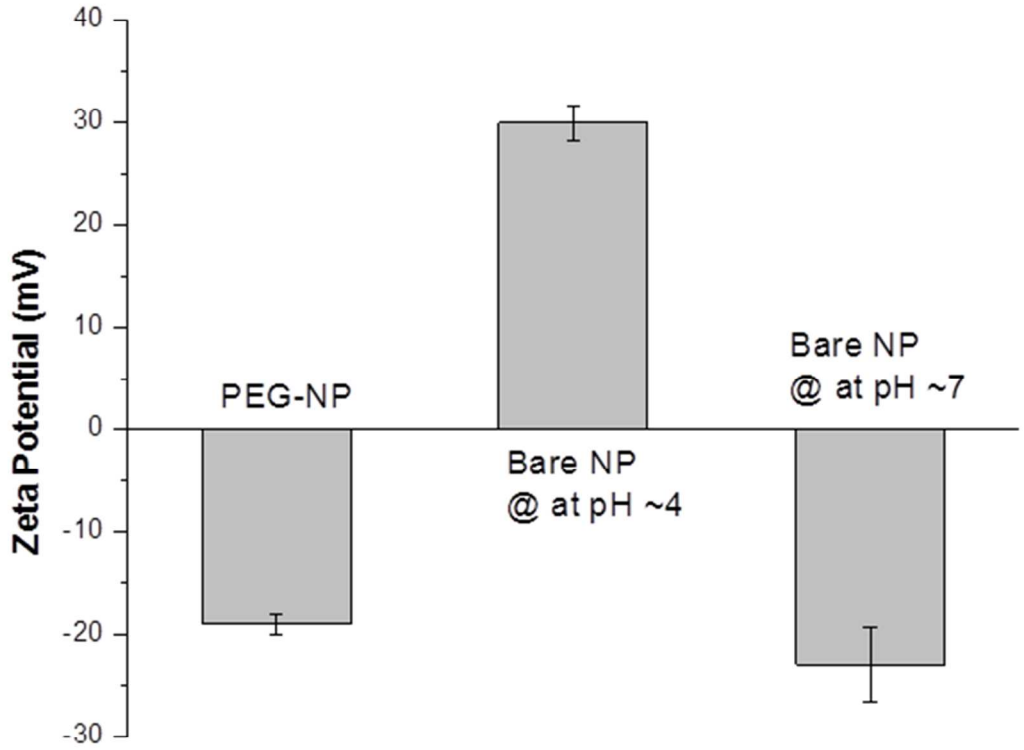
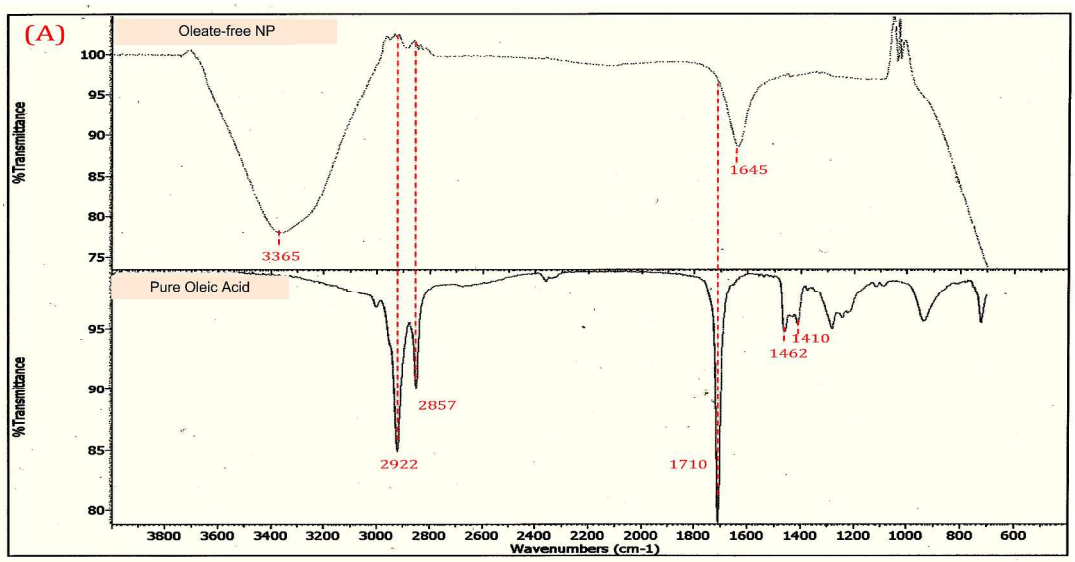
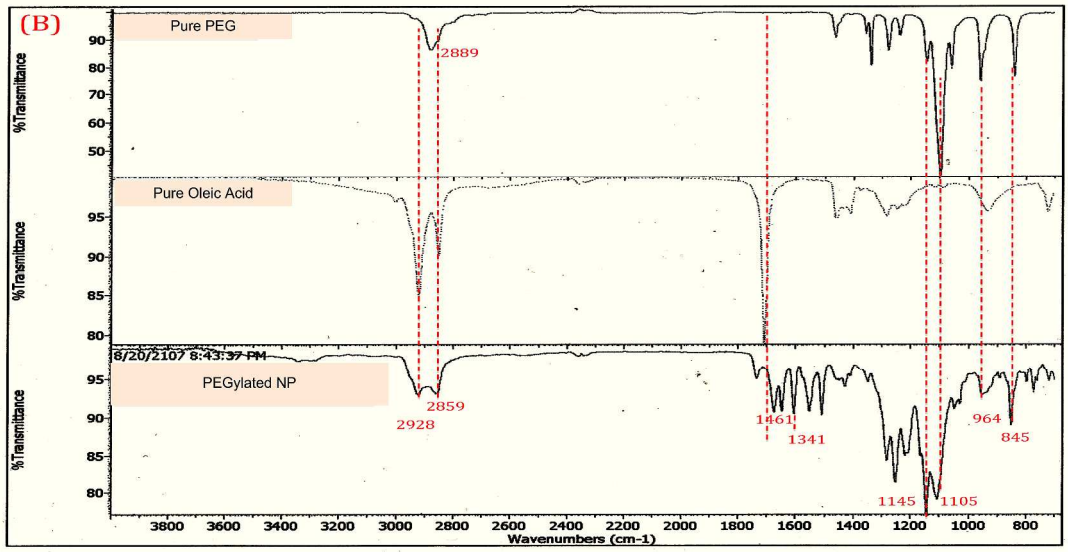


Figure S2. Zeta-potential of the PEG-oleate bilayered NPs and ligand free NPs at reaction condition (pH ~4) and condition at cell study (pH ~7).


(A)
Oleate-free NP
%Transmittance
3365
1645
Pure Oleic Acid
2922
2857
1710
1462
1410
Wavenumbers (cm-1)


(B)
Pure PEG
2889
Pure Oleic Acid
8/20/2107 8:43:37 PM
PEGylated NP
2928
2859
1461
1341
1145
1105
964
845
%Transmittance
Wavenumbers (cm-1)

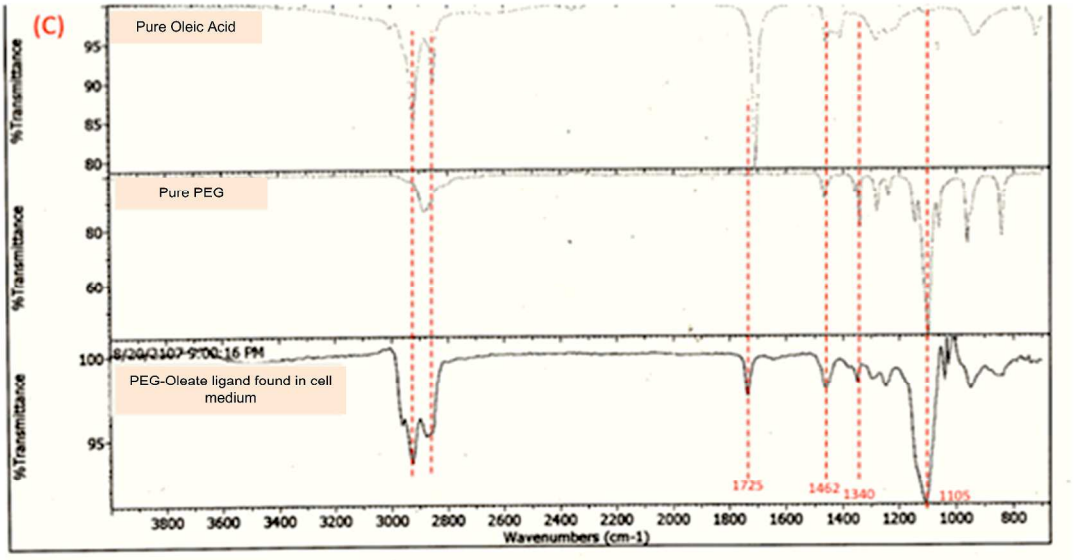

(C)
Pure Oleic Acid
Pure PEG
8/20/2107 9:00:16 PM
PEG-Oleate ligand found in cell medium
1725
1462
1340
1105
%Transmittance
Wavenumbers (cm-1)

Figure S3. Fourier transformation infrared (FTIR) spectra of: (A) bare surface $NaYF_4$:Yb,Er (i.e. ligand free) NPs and comparison with pure oleic acid, (B) typical PEGylated $NaYF_4$:Yb,Er NPs (i.e. both PEG-oleate bilayered and covalently bound PEG-NPs showed similar spectra) and comparison with pure oleic acid and pure PEG, and (C) the PEG-oleate ligand molecules found in cell media.

To characterize the surface-treated nanoparticles, FTIR spectra were collected. Figure S3 represents the spectra of oleic acid, PEG, PEG-oleate intercalated NPs and PEG-monooleate molecules found in the cell culture medium. The assignments of peaks are listed in the Table S1. Fig. S3(A) shows the comparison of pure oleic acid and olate-stripped bare particles (i.e. ligand free) particle. As shown in the figure the characteristic peaks of oleic acid at 2922, 2857 and 1710 cm^{-1} due to asymmetric, symmetric CH_2 stretch, and C=O stretch respectively are absent in the oleate free NPs (i.e. bare particle). Two peaks appeared at 3365 cm^{-1} and 1645 cm^{-1} are originated from O-H stretching vibration and bend vibration of surface hydroxyl groups of the stripped particles. Fig. S3 (B) shows the spectra of the PEG-oleate intercalated particles where the CH_2 stretching bands of oleic acid is little broad due to CH_2 stretching band of PEG at 2889 cm^{-1}. The band at 1710 cm^{-1} is suppressed due to other bands of PEG at 1461 cm^{-1} and around. The intense peak for C-O stretching of PEG shows up at 1105- 11045 cm^{-1} in the intercalated NPs. However, when we found and separated PEG-mono-oleate molecules in the cell culture media, they show neat peaks of PEG and oleic acids as shown in Fig. S3 (C).

Table S1. Assignments of FTIR peaks of the sample

	Wavenumber (cm^{-1})	**Assignments**	**Reference**
Oleic acid	2922	asymmetric CH_2 stretch	Wu et al.[S1]
	2857	symmetric CH_2 stretch	
	1710	C=O stretch	
	1462	In plane O-H band	
	1410	CH_3 umbrella mode	
PEG	2889	CH_2 stretch	Lin et al.[S2]
	1461	CH_2 bending	
	1341	OH bending, CH_2 formation	
	1105	C-O stretching	
	964	CH_2 bending	
	845	C-C stretching	

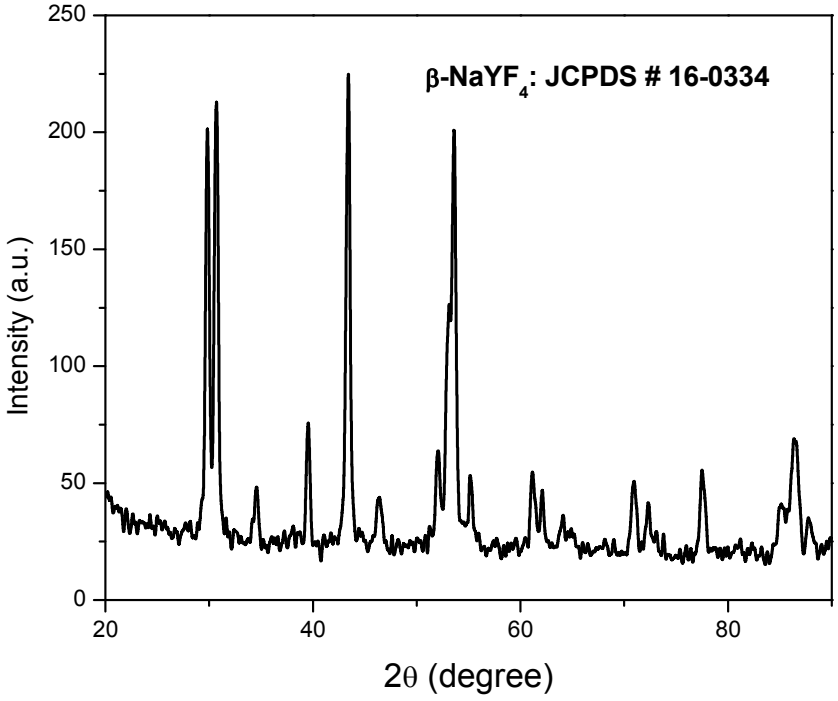


Figure S4. XRD pattern of the synthesized $NaYF_4$:Yb,Er

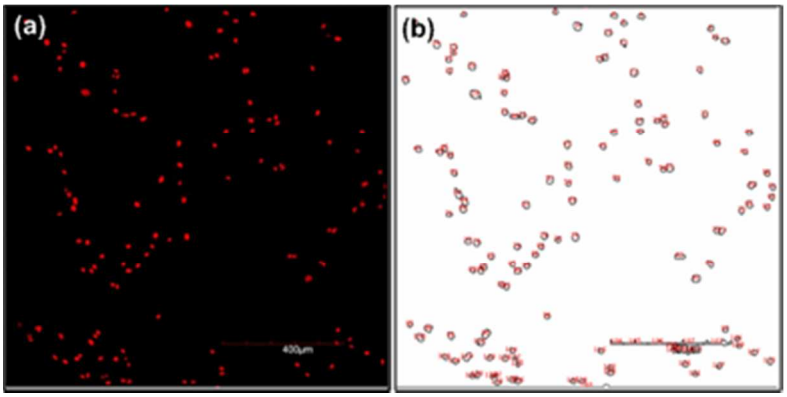


Figure S5. Propidium iodide (PI) stained control cells and corresponding image J analysis.

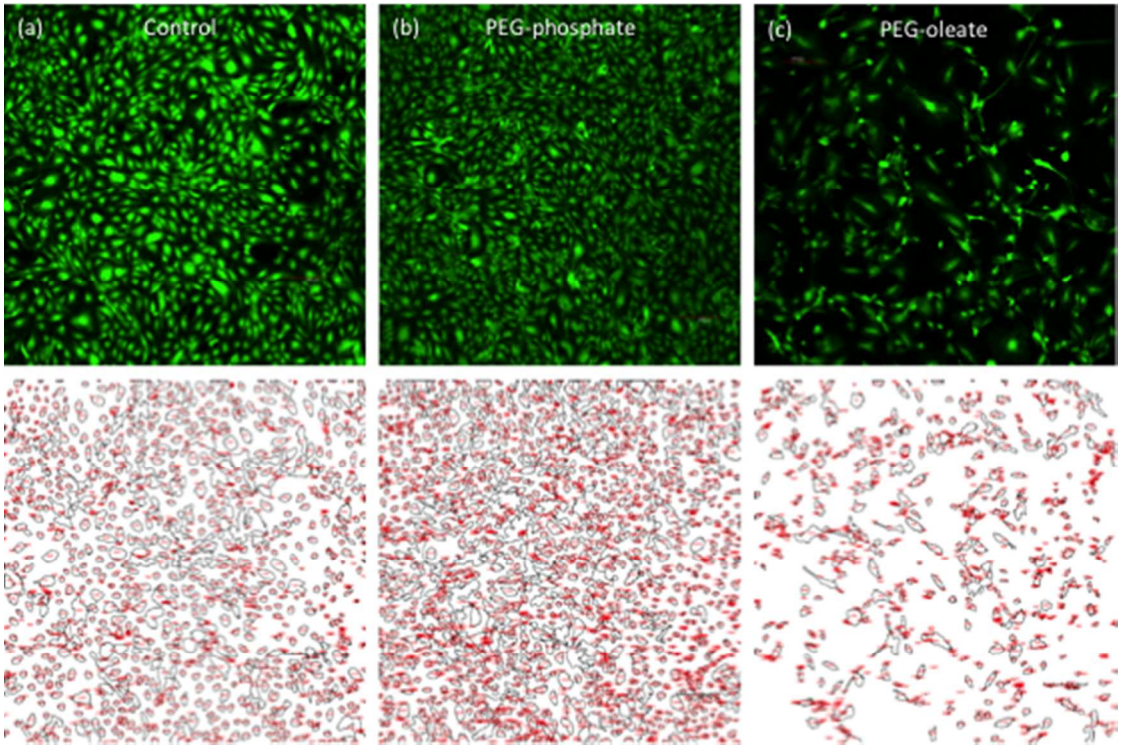


Figure S6. Fluorescent image of cells from calcein assays (top panel), and quantification analysis of the confocal fluorescent images using Image J analysis (bottom). All cells were incubated with 75 μg/ml NPs for 24 h. Surface area counts were normalized by calcein control. Analysis shows that the HAEC control has 1426 cells (28.6% of area) while covalently bound PEG-NPs have 2312 cells (32.2% of area) compared to PEG-oleate bilayered NPs which have 1235 cells (11.0% area)

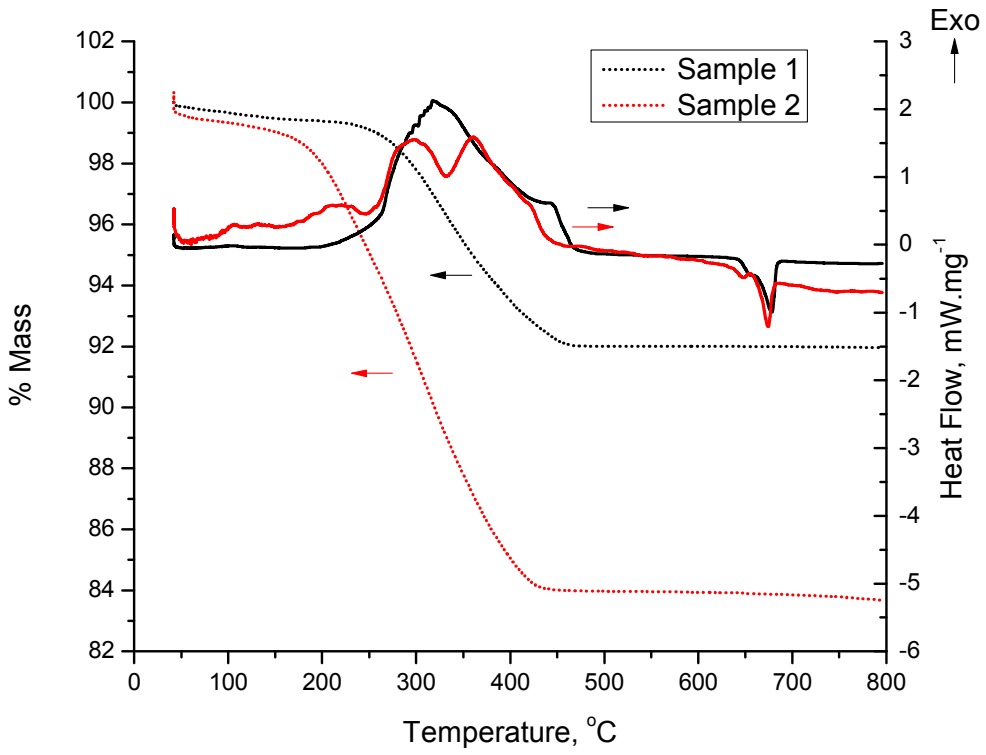


Figure S7. Thermogravimetric analysis (TGA) of oleate capped $NaYF_4$;Yb,Er and PEG-oleate bilayered $NaYF_4$;Yb,Er NPs. (Sample 1 being oleate-capped $NaYF_4$;Yb,Er, and sample 2 being PEG-oleate bilayered $NaYF_4$;Yb,Er NPs)

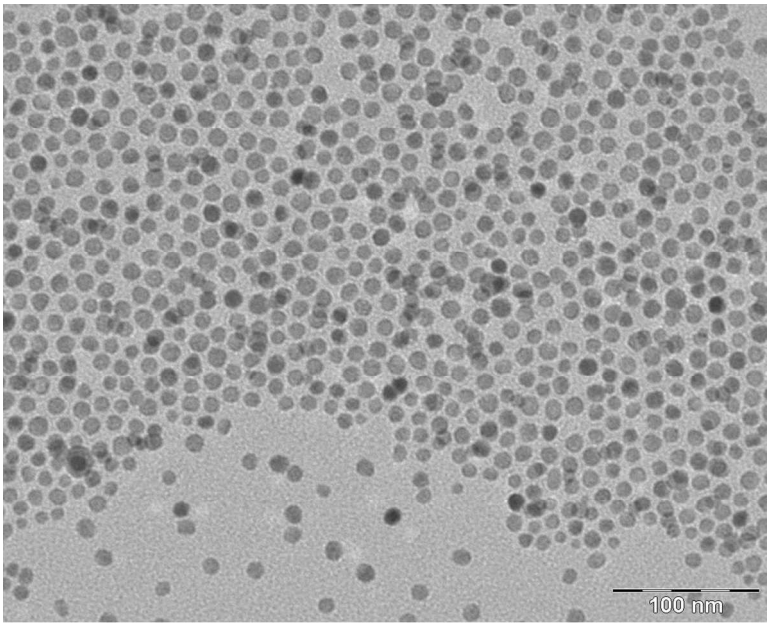


Figure S8. TEM micrograph of $NaGdF_4$;Yb,Er used in the study. Average particle size was measured to be ~13 nm.

REFERENCES

[S1] Wu N, Fu L, Su M, Aslam M, Wong KC, Dravid VP. Interaction of Fatty Acid Monolayers with Cobalt Nanoparticles. Nano Letters. 2004;4:383-6.

[S2] Lin Z, Han X, Wang T, Li S. Effects of adding nano metal powders on thermooxidative degradation of poly(ethylene glycol). J Therm Anal Calorim. 2008;91:709-14.

[S3] Changfu X, Mo M, Liwen Y, Songjun Z, Qibin Y. Upconversion luminescence and magnetic properties of ligand-free monodisperse lanthanide doped $BaGdF_5$ nanocrystals. J Lumin. 2011; 131; 2544 – 49.