

## Supporting Information

### **Fluorescent polypropylene nanoplastics for studying uptake, biodistribution, and excretion in zebrafish embryos**

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## **Experimental section**

### **Quantitative analysis of R-PPNPs in zebrafish embryos (ZFEs)**

We employed the fluorescence intensity of RBITC-labeled PPNPs (R-PPNPs) in a quantitative relationship model to determine the amount of R-PPNPs remaining in ZFEs. To do so, ZFEs were treated with 50 ppm R-PPNPs at 72 hpf for 24 h and then washed 3 times with fresh EW and fixed with 4% paraformaldehyde at 96 hpf and 120 hpf. Each ZFE was homogenized, and the fluorescence intensity was measured. The amounts of R-PPNPs in ZFEs were calculated by adjusting the standard curve.

Table S1. The yield of PPNPs production by NIPS method

Number	Mass (mg)		Yield (%)	Average
	In	Out		
1	130	100	76.9%	84.1%
2	130	100	76.9%	
3	260	230	88.5%	
4	1040	980	94.2%	

Table S2. DSC melting results of PP pellets and PPNPs

<b>Samples</b>	<b>T<sub>c</sub> (°C)</b>	<b>T<sub>m-low</sub> (°C)</b>	<b>T<sub>m-high</sub> (°C)</b>	<b>ΔH<sub>f</sub> (J/g)</b>	<b>X<sub>DSC</sub> (%)</b>
PP pellets	115.40	147.53	156.52	102.39	49.44
PPNPs	120.87	149.54	156.64	105.31	50.85

Table S3. Mortality and deformity of PPNPs and R-PPNPs-treated ZFEs ( $P > 0.05$ )

Conc. (ppm)		Mortality rate (%)		Deformity rate (%)	
		at 48 hpf	at 96 hpf	at 48 hpf	at 96 hpf
R-PPNPs	0	13.33 ± 5.77	0.00	3.33 ± 5.77	0.00
	50	13.33 ± 5.77	0.00	6.66 ± 5.77	0.00
PPNPs	0	6.66 ± 5.77	0.00	0.00	0.00
	50	0.00	0.00	3.33 ± 5.77	0.00

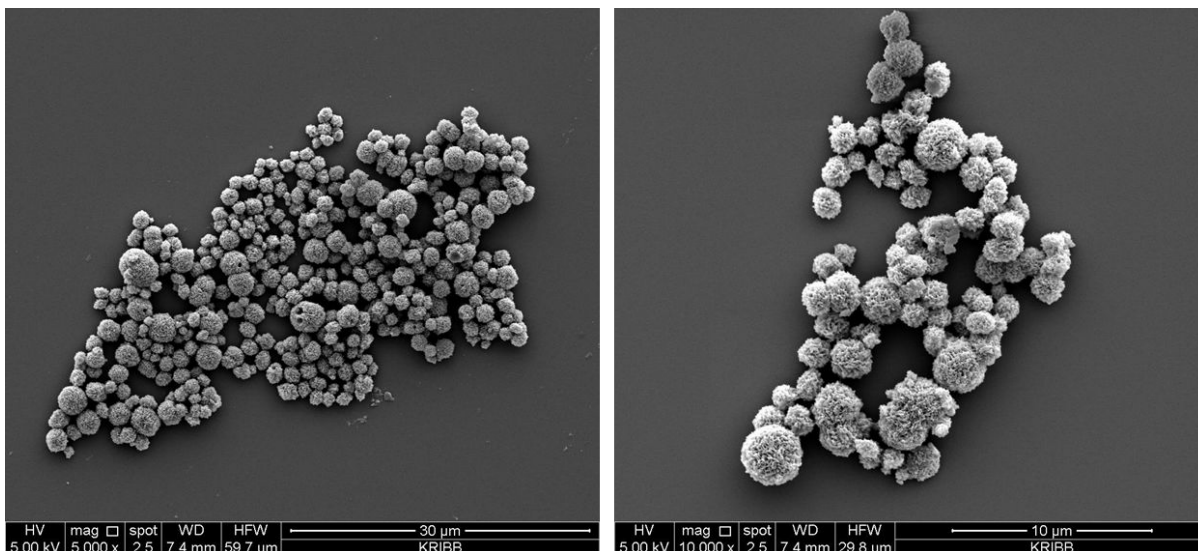


Figure S1. SEM images of PPNPs prepared by NIPS method using distilled water as a non-solvent.

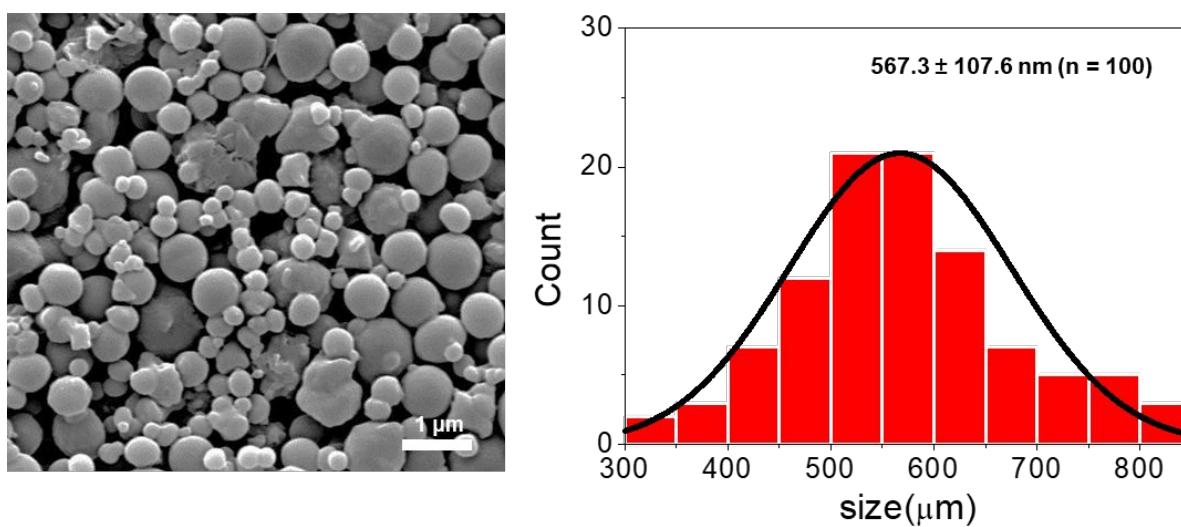


Figure. S2. SEM images of R-PPNPs and its size distribution.

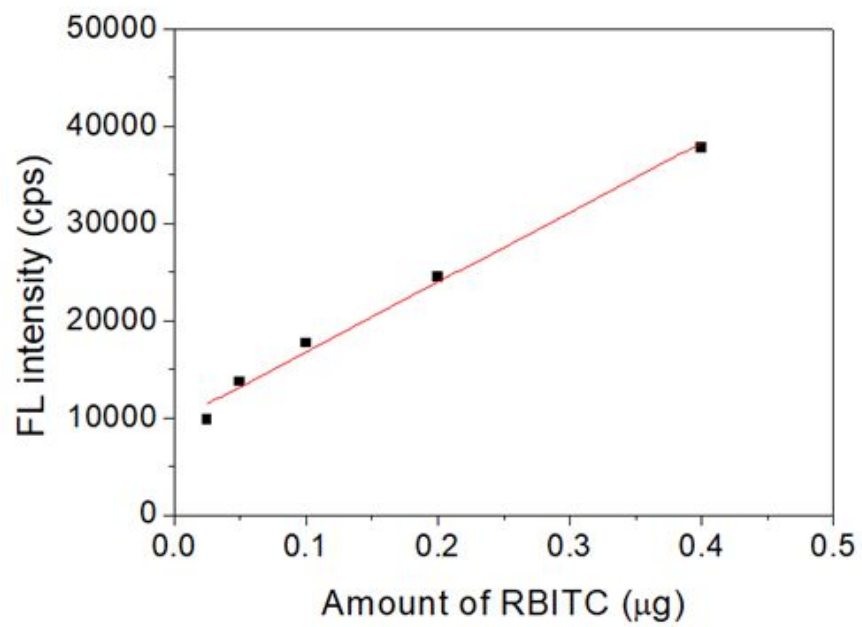


Figure S3. Standard curve for fluorescence intensity of RBITC by concentration.

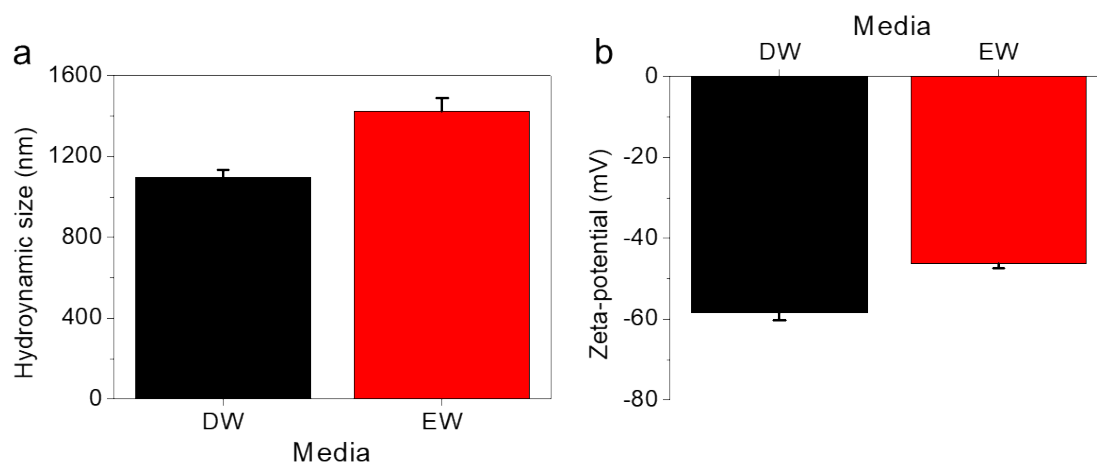


Figure S4. (a) Hydrodynamic size and (b) Zeta-potential values of PPNPs by different media (DW and EW)



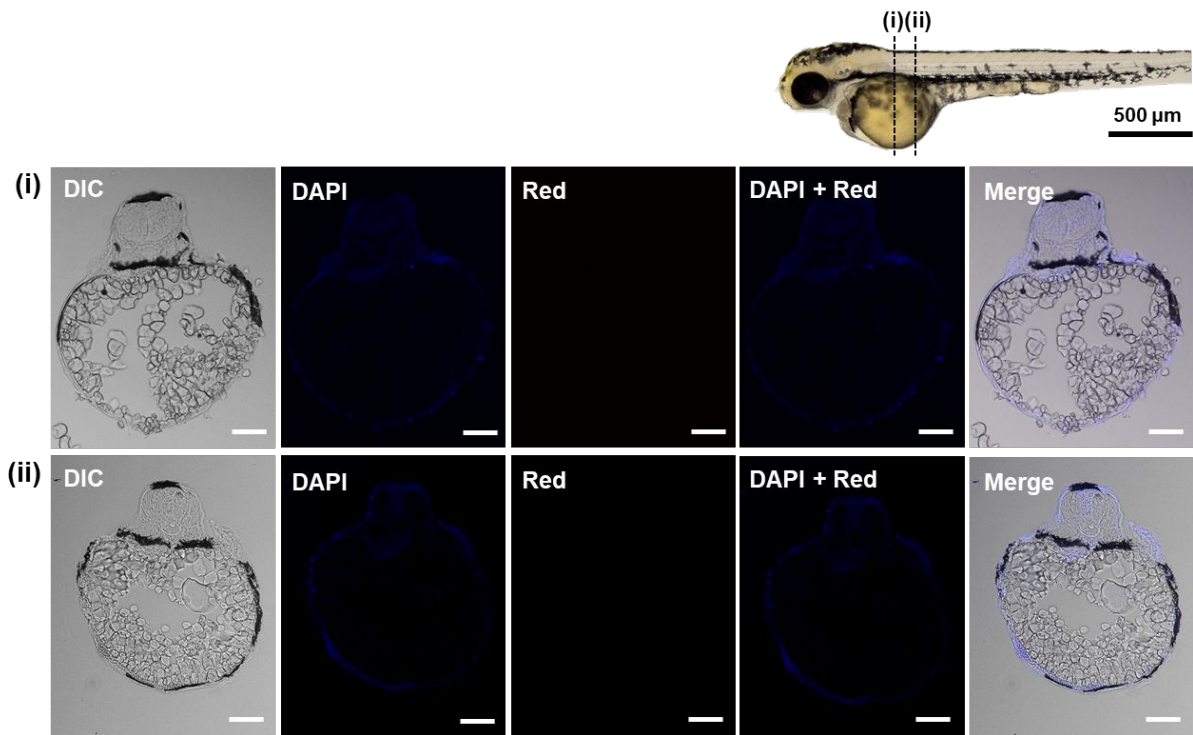


Figure S5. Cross-sectional images of the yolk sac (The point of dash line in top image i , ii ) in ZFEs at 48 hpf after R-PPNPs exposure at 24 hpf for 24 h with three different filters such as DIC, DAPI, and Red.

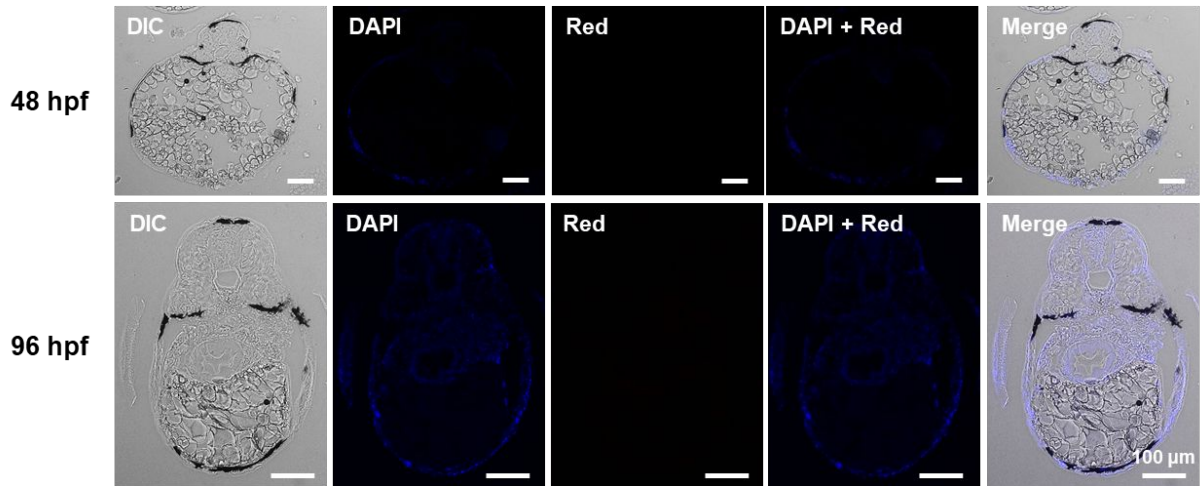
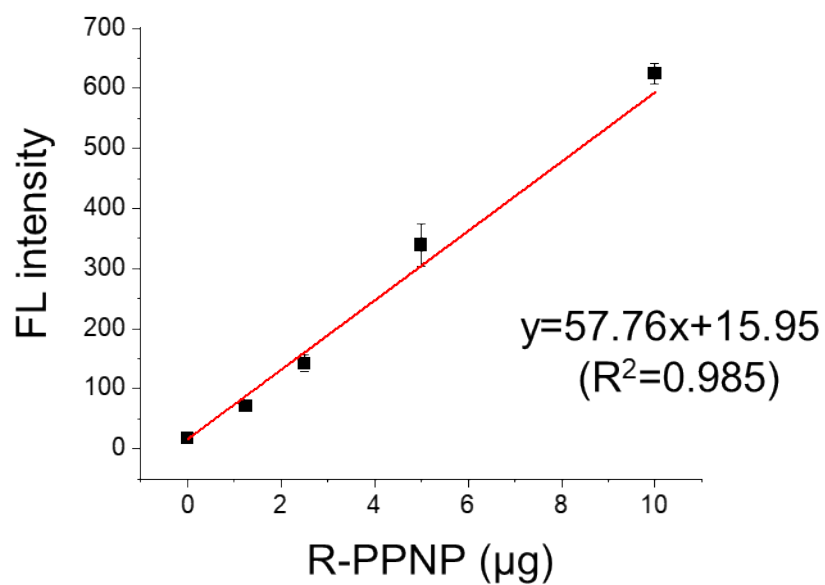


Figure S6. Cross-sectional images of control ZFEs at 48 hpf and 96 hpf with three different filters such as DIC, DAPI, and Red.



(n=5)	96 hpf	120 hpf
Control	0.00 ± 0.05 µg/individual	0.00 ± 0.00 µg/individual
R-PPNP	1.01 ± 0.09 µg/individual	0.46 ± 0.10 µg/individual

Figure S7. Standard curve and amount of R-PPNPs measured by fluorescent microplate reader (Cytation 5, BioTek, USA) under excitation/emission at 540/580 nm.