

## High-Load Nanoparticles with Chemotherapeutic SN-38/FdUMP Drug Cocktail

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## 1. Analytical Equipment

*Scanning electron microscopy (SEM)* was carried out with a Zeiss Supra 40 VP (Zeiss, Germany), equipped with a field emission gun (acceleration voltage 5 kV, working distance 3 mm). Samples were prepared by spraying a diluted aqueous suspension of the as-prepared IOH-NPs with a mist maker on a silica wafer that was left for drying overnight.

*Energy-dispersive X-ray spectroscopy (EDXS)* was performed with an Ametek EDAX device (Ametek, USA) mounted on the above described Zeiss SEM Supra 40 VP scanning electron microscope. For the analysis, the IOH-NPs were dried at 50 °C and thereafter pressed to dense pellets in order to guarantee for a smooth surface and a quasi-infinite layer thickness. These pellets were fixed with conductive carbon pads on aluminium sample holders. An acceleration voltage of 30 kV was used for these measurements.

*Dynamic light scattering (DLS)* and *zeta potential* measurements were carried out on a Zetasizer Nano-ZS (Malvern, United Kingdom) with a 633 nm laser and backscattering geometry (173°). For DLS measurements, the aqueous IOH-NP suspensions were diluted 1 : 10 or 1 : 20 with demineralized water. The zeta potential of the as-prepared IOH-NPs was also measured using these diluted aqueous suspensions.

*Fourier-transform infrared (FT-IR)* spectra were recorded on a Bruker Vertex 70 FT-IR spectrometer (Bruker, Germany) in the range from 4000 to 400 cm<sup>-1</sup> with a resolution of 4 cm<sup>-1</sup>. For this purpose, 1 mg of dried IOH-NPs was pestled with 300 mg of KBr and pressed to a pellet, which thereafter was measured in transmission.

*Differential thermal analysis/thermogravimetry (DTA/TG)* was performed with a STA409C device (Netzsch, Germany). The measurements were performed in air to guarantee for total combustion of the organic content. The IOH-NPs (20 mg in corundum crucibles), pre-dried at 100 °C for 5 h, were heated to 1200 °C with a rate of 5 K/min.

*X-ray diffraction (XRD)* measurements of the as-prepared IOH-NPs as well as of the residue after total organic combustion of these IOH-NPs (TG analysis) were performed with a Stadi MP diffractometer (STOE & Cie, Germany) using a Cu-K<sub>α1</sub> radiation source ( $\lambda = 154.05$  pm) and a germanium-(111)-monochromator.

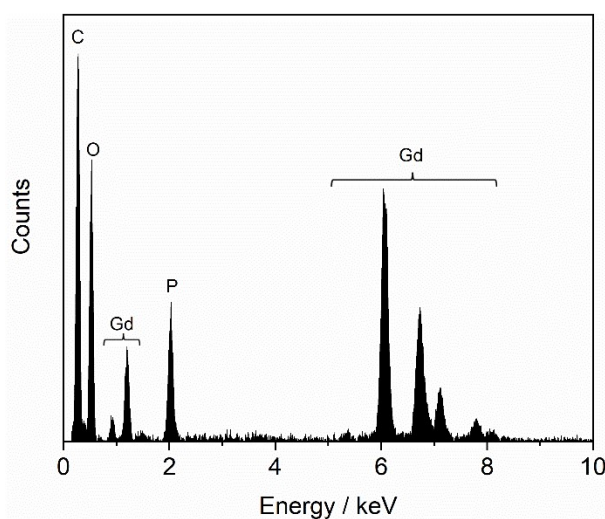
*Elemental analysis (EA, C/H/N/S analysis)* was performed via thermal combustion with an Elementar Vario Microcube device (Elementar, Germany) at a temperature of 1150 °C. The samples were pre-dried at 100 °C for 5 h to remove the remaining solvent.

*Photoluminescence (PL)* measurements were performed with a Horiba Jobin Yvon Spex Fluorolog 3 (Horiba Jobin Yvon, France) equipped with a 450 W Xe-lamp and double-grating excitation and emission monochromators.

## 2. Material Characterisation $[\text{Gd}(\text{OH})]^{2+}[(\text{SN-38})_{0.5}(\text{UMP})_{0.5}]^{2-}$ IOH-NPs

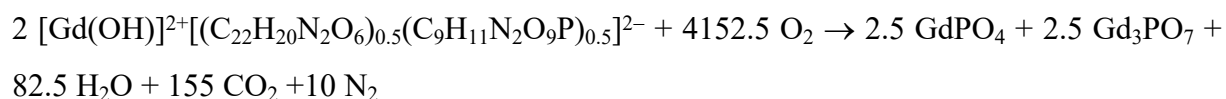
$[\text{Gd}(\text{OH})]^{2+}[(\text{SN-38})_{0.5}(\text{UMP})_{0.5}]^{2-}$  are used as representatives for drug-cocktail containing  $[\text{Gd}(\text{OH})]^{2+}[(\text{SN-38})_{0.5}(\text{FdUMP})_{0.5}]^{2-}$ . The chemotherapeutic FdUMP differentiates from UMP by a fluorine atom in 5'-position of the uridine unit (instead of hydrogen) and a hydrogen atom (instead a hydroxyl group at the 2' carbon of the ribose unit). FdUMP, however, is by a factor 800× more expensive (i.e., 4,000 € for 100 mg  $\text{Na}_2(\text{FdUMP})$  but about 5 € for 100 mg  $\text{Na}_2(\text{UMP})$ ). Therefore, all syntheses and characterization were performed with  $[\text{Gd}(\text{OH})]^{2+}[(\text{SN-38})_{0.5}(\text{UMP})_{0.5}]^{2-}$ .  $[\text{Gd}(\text{OH})]^{2+}[(\text{SN-38})_{0.5}(\text{FdUMP})_{0.5}]^{2-}$  was only used to perform the *in vitro* experiments. In terms of a characterization of the particle size and chemical composition,  $[\text{Gd}(\text{OH})]^{2+}[(\text{SN-38})_{0.5}(\text{UMP})_{0.5}]^{2-}$  and  $[\text{Gd}(\text{OH})]^{2+}[(\text{SN-38})_{0.5}(\text{FdUMP})_{0.5}]^{2-}$  can be considered to be similar (except for fluorine and hydroxyl group).

Energy dispersive X-ray spectroscopy (EDXS) of the  $[\text{Gd}(\text{OH})]^{2+}[(\text{SN-38})_{0.5}(\text{UMP})_{0.5}]^{2-}$  IOH-NPs qualitatively proves the presence of gadolinium and phosphorus (Figure S1).

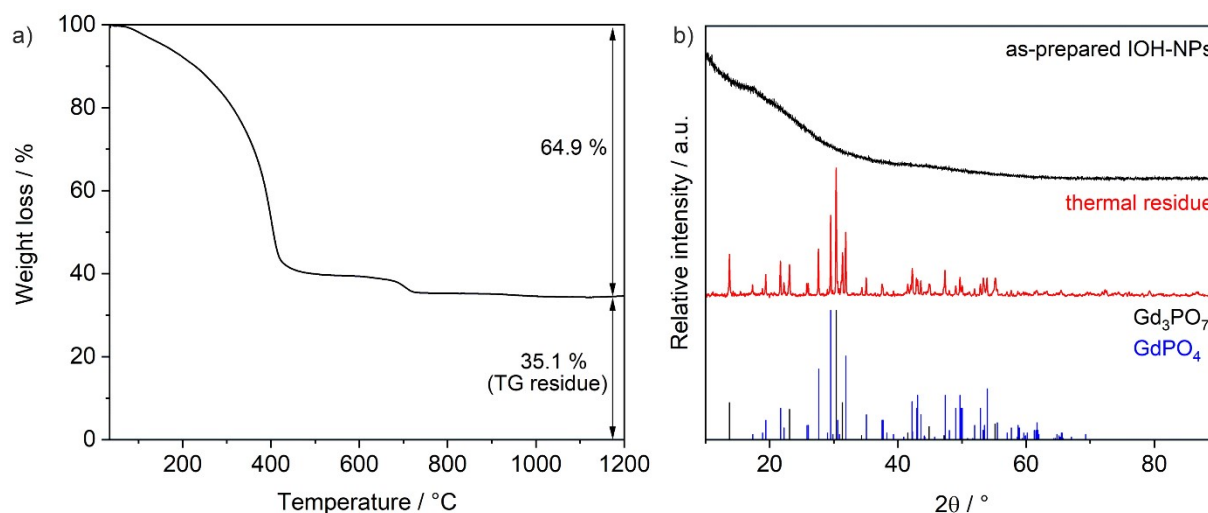


**Figure S1.** EDX analysis of  $[\text{Gd}(\text{OH})]^{2+}[(\text{SN-38})_{0.5}(\text{UMP})_{0.5}]^{2-}$  IOH-NPs.

Total organics combustion of the  $[\text{Gd}(\text{OH})]^{2+}[(\text{SN-38})_{0.5}(\text{UMP})_{0.5}]^{2-}$  IOH-NPs was performed by thermogravimetry (TG) (Figure S2a; see main paper: Table 1). The thermal decomposition of the IOH-NPs can be rationalized based on the following reaction:



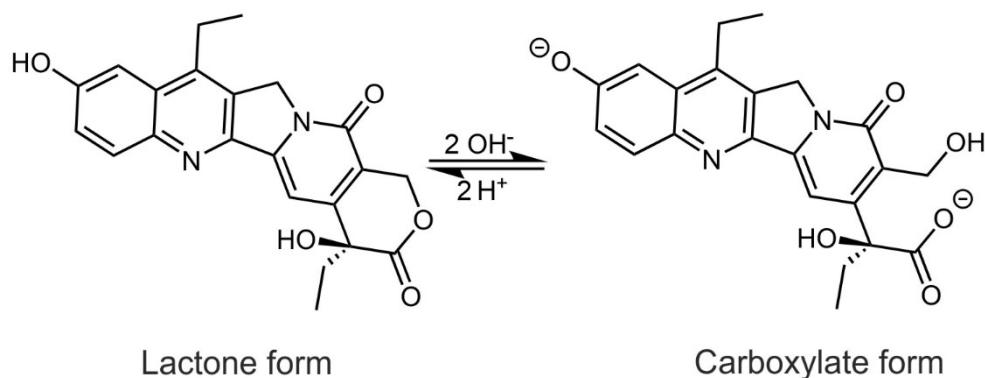
The presence of  $\text{Gd}_3\text{PO}_7$  and  $\text{GdPO}_4$  as remnants of the thermal decomposition was evidenced by X-ray powder diffraction (XRD) (Figure S2b).



**Figure S2.** Thermal properties of  $[\text{Gd}(\text{OH})]^{2+}[(\text{SN-38})_{0.5}(\text{UMP})_{0.5}]^{2-}$  IOH-NPs: a) TG; b) XRD of the thermal remnant after TG analysis ( $\text{Gd}_3\text{PO}_7$ /ICDD-No. 00-034-1066,  $\text{GdPO}_4$ /00-032-0386 as references).

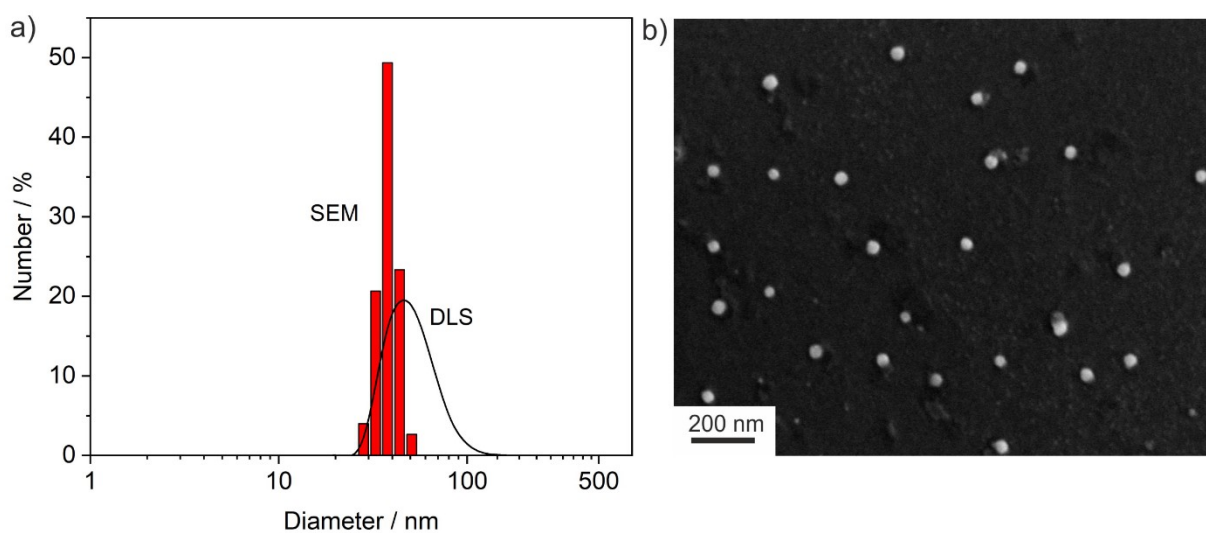
### 3. Material Characterisation $[\text{Gd}(\text{OH})]^{2+}[\text{SN-38}]^{2-}$ IOH-NPs

SN-38 is known for its reversible pH-dependent equilibrium between the therapeutically active lactone form and the inactive carboxylate form in aqueous to alkaline solution ( $\text{pH} \geq 7$ ) (Figure S3).<sup>S1</sup> In contrast to the closed lactone form, the open carboxylate form is soluble in water starting at a pH of 8. In order to verify the formation of IOH-NPs with SN-38,  $[\text{Gd}(\text{OH})]^{2+}[\text{SN-38}]^{2-}$  IOH-NPs with only SN-38 as an anion were prepared and analysed in addition to the  $[\text{Gd}(\text{OH})]^{2+}[(\text{SN-38})_{0.5}(\text{UMP})_{0.5}]^{2-}$  IOH-NPs.



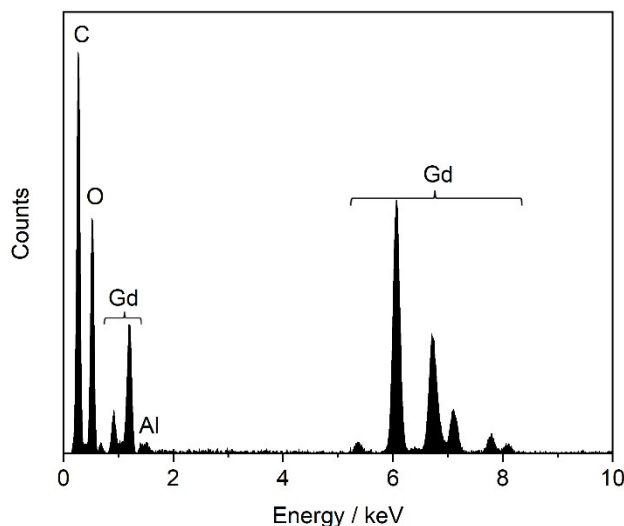
**Figure S3.** Molecular structure of SN-38 with pH-dependent equilibrium.

Particle size, size distribution and colloidal stability of the  $[\text{Gd}(\text{OH})]^{2+}[\text{SN-38}]^{2-}$  IOH-NPs were characterized by dynamic light scattering (DLS) and scanning electron microscopy (SEM). DLS of suspensions in diethylene glycol indicates a mean hydrodynamic diameter of  $51 \pm 14$  nm (Figure S4a). DEG was here used as a polar solvent, showing less extensive hydrogen bridging than water. As a result, the hydrodynamic diameters originating from DLS is much more comparable to the mean diameter stemming from electron microscopy. SEM confirms the presence of spherical particles with a mean diameter of  $38 \pm 4$  nm (based on statistical evaluation of  $> 100$  nanoparticles on SEM images) (Figure S4a,b).

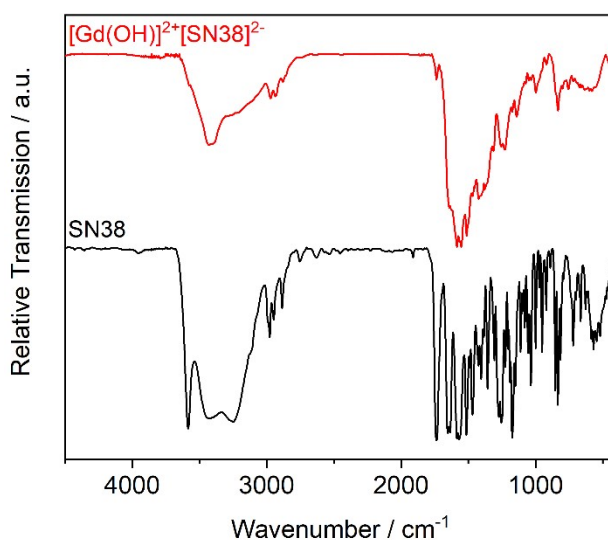


**Figure S4.** Particle features of  $[\text{Gd}(\text{OH})]^{2+}[\text{SN-38}]^{2-}$  IOH-NPs: a) particle size and size distribution according to DLS (in DEG) and SEM; b) SEM overview image.

EDXS of the  $[\text{Gd}(\text{OH})]^{2+}[\text{SN-38}]^{2-}$  IOH-NPs qualitatively proves the presence of gadolinium (Figure S5).



**Figure S5.** EDX analysis of  $[\text{Gd}(\text{OH})]^{2+}[\text{SN-38}]^{2-}$  IOH-NPs.

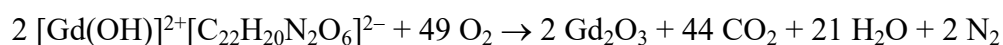


**Figure S6.** FT-IR spectrum of  $[\text{Gd}(\text{OH})]^{2+}[\text{SN-38}]^{2-}$  IOH-NPs (with pure SN-38 as reference).

The presence of SN-38 in the  $[\text{Gd}(\text{OH})]^{2+}[\text{SN-38}]^{2-}$  IOH-NPs is validated by Fourier-transform infrared (FT-IR) spectroscopy. Thus, the observed vibrations are similar to pure SN-38 as a reference (Figure S6). In comparison to the fine-splitted vibrations of pure SN-38, the IOH-NPs show broader vibrations, enveloping the fine-splitted vibrations of pure SN-38. This finding can be ascribed to non-crystallinity of the IOH-NPs and a great number of individual anions whose vibrational energy is slightly different to each other (Figure S6). Especially, the most intense vibration of pure SN-38 ( $1735\text{ cm}^{-1}$ ) should be noticed as it indicates the lactone ring in its closed form. This vibration vanishes almost completely for the IOH-NPs and proves the presence of the open carboxylate form in the IOH-NPs (Figure S6). In contrast, the most intense vibration of the  $[\text{Gd}(\text{OH})]^{2+}[\text{SN-38}]^{2-}$  IOH-NPs is observed at  $1585\text{ cm}^{-1}$  and can be

assigned to  $\nu(\text{COO})$  of the open carboxylate form. Moreover, vibrations at 3500-3000 ( $\nu(\text{O-H})$ ) and 2970-2850  $\text{cm}^{-1}$  ( $\nu = (\text{C-H})$ ) as well as the fingerprint area  $< 1500 \text{ cm}^{-1}$ ) of the  $[\text{Gd}(\text{OH})]^{2+}[\text{SN-38}]^{2-}$  IOH-NPs are well in agreement with the reference spectrum.

In addition to the qualitative analysis of the presence of  $\text{Gd}^{3+}$  (EDX) and  $[\text{SN-38}]^{2-}$  (FT-IR), the chemical composition of the  $[\text{Gd}(\text{OH})]^{2+}[\text{SN-38}]^{2-}$  IOH-NPs was quantified by elemental analysis (EA) and total-organics combustion via thermogravimetry (TG). A comparison of the experimental and calculated values indicates the experimental N/C/H values and the total-organics combustion to be systematically too low (Table S1). Here, it must be taken into account that the nucleation of  $[\text{Gd}(\text{OH})]^{2+}[\text{SN-38}]^{2-}$  was performed with slightly alkaline conditions ( $\text{pH} = 8$ ) in order to keep SN-38 in its open carboxylate form. This also results in a deprotonation of the phenol group of SN-38, leading to the divalent anion  $[\text{SN-38}]^{2-}$ . Because of the alkaline conditions, however, certain amount of  $\text{Gd}(\text{OH})_3$  can be formed as well, which is the reason for the reduced amount of SN-38 in the IOH-NPs. Based on a co-precipitation of  $[\text{Gd}(\text{OH})]^{2+}[\text{SN-38}]^{2-}$  with 13 wt-% of  $\text{Gd}(\text{OH})_3$ , the experimental results of EA and TG are in good accordance with the calculated values (Table S1, Figure S7a). Total organics combustion can be rationalized based on the following reaction:

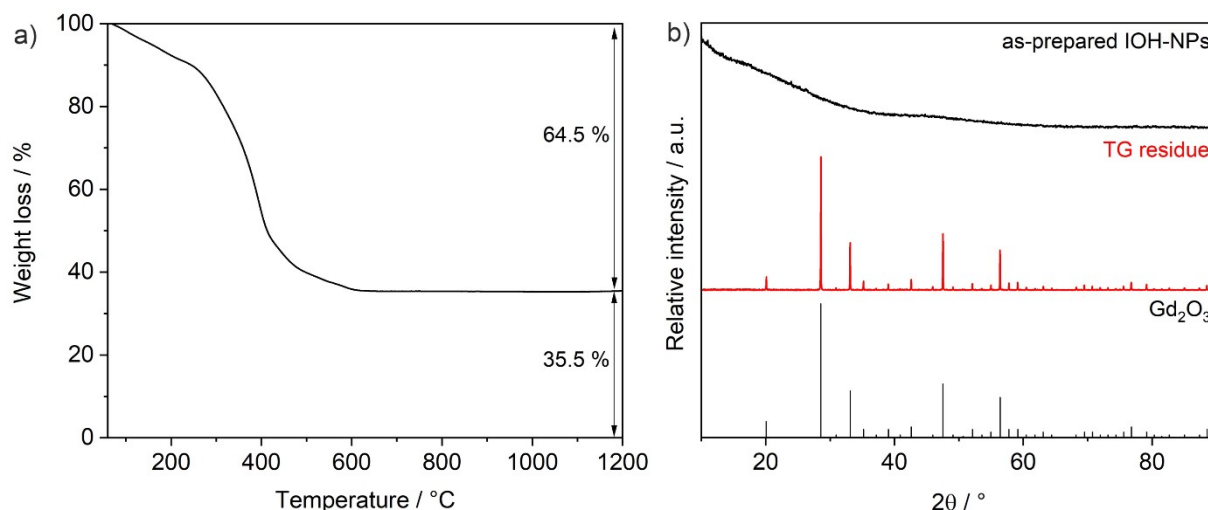


The presence of  $\text{Gd}_2\text{O}_3$  as the thermal remnant was evidenced by XRD (Figure S7b).

In contrast to the  $[\text{Gd}(\text{OH})]^{2+}[\text{SN-38}]^{2-}$  IOH-NPs, no co-precipitation of  $\text{Gd}(\text{OH})_3$  was observed for  $[\text{Gd}(\text{OH})]^{2+}[(\text{SN-38})_{0.5}(\text{UMP})_{0.5}]^{2-}$  IOH-NPs (*see main paper: Table 1*), which can be ascribed to the additional presence of the phosphate-functionalized  $[\text{UMP}]^{2-}$  forming a stronger bond and less soluble compound with  $\text{Gd}^{3+}$  than  $\text{OH}^-$ .

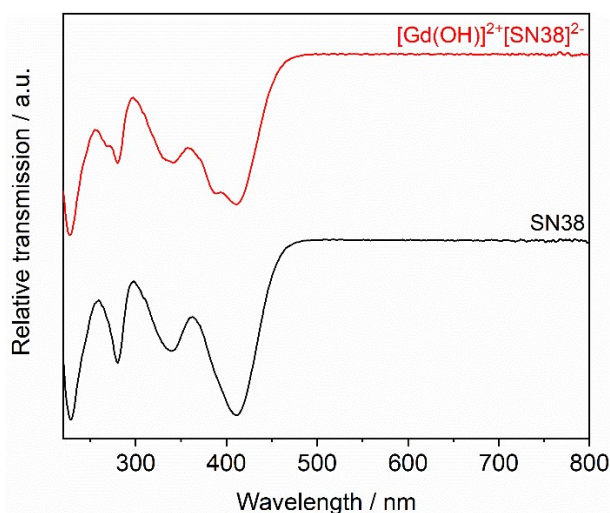
**Table S1.** Composition of the as-prepared IOH-NPs with 83 %  $[\text{Gd}(\text{OH})]^{2+}[\text{SN-38}]^{2-}$  and 13 %  $\text{Gd}(\text{OH})_3$  according to EA and TG.

	EA contents			TG mass loss
	N / wt-%	C / wt-%	H / wt-%	Weight loss / wt-%
<b>Experimental</b>	3.4	34.7	3.2	64.5
(corrected for 13 wt-% $\text{Gd}(\text{OH})_3$ )	4.5	39.2	3.6	72.8
<b>Calculated</b>	4.8	45.4	3.4	68.9



**Figure S7.** Thermal properties of  $[\text{Gd}(\text{OH})]^{2+}[\text{SN-38}]^{2-}$  IOH-NPs: a) TG, b) XRD of the thermal remnant after TG analysis ( $\text{Gd}_2\text{O}_3$  ICDD-No. 00-021-0797 as a reference).

Optical spectroscopy (UV-Vis) of the  $[\text{Gd}(\text{OH})]^{2+}[\text{SN-38}]^{2-}$  IOH-NPs further confirms the presence of SN-38 in the open carboxylate form. Thus, the characteristic absorption below 450 nm is observed for the IOH-NPs as well as for alkaline solutions of SN-38 (pH = 8) (Figure S8). Especially the absorptions at 335 and 410 nm are characteristic of the open carboxylate form of SN-38.<sup>S2</sup> These absorptions are also in accordance with the yellowish colour of the  $[\text{Gd}(\text{OH})]^{2+}[\text{SN-38}]^{2-}$  IOH-NPs as well as of alkaline solutions of SN-38.

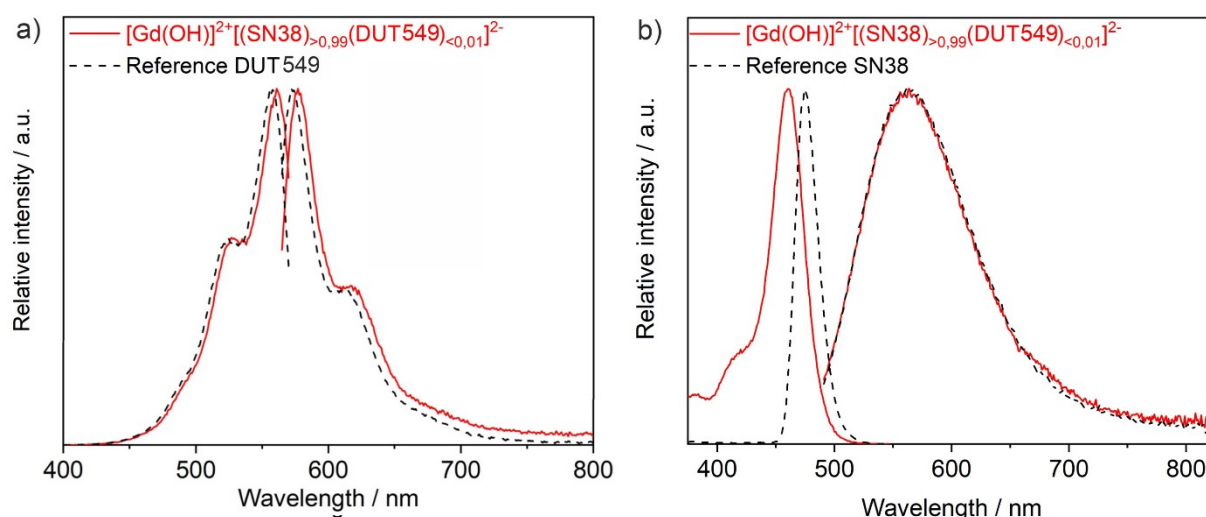


**Figure S8.** UV-Vis spectrum of  $[\text{Gd}(\text{OH})]^{2+}[\text{SN-38}]^{2-}$  IOH-NPs (solution of SN-38 at pH 8 as a reference).

Similar to the  $[\text{Gd}(\text{OH})]^{2+}[(\text{SN-38})_{0.5}(\text{FdUMP})_{0.5}]^{2-}$  IOH-NPs (see main paper: Figure 3b), the  $[\text{Gd}(\text{OH})]^{2+}[\text{SN-38}]^{2-}$  IOH-NPs can be fluorescence-labelled by addition of minor amounts



(0.01 mol-%) of Dyomics DY-549-dUTP (DUT549). These DUT549-labelled  $[\text{Gd}(\text{OH})]^{2+}[\text{SN-38}]^{2-}$  IOH-NPs show red emission at 570-700 nm ( $\lambda_{\text{max}} = 585$  nm) upon excitation at 460-570 nm, which is similar to pure DUT549 in solution as a reference (Figure S9a). In addition, it should be noticed that SN-38 also shows emission itself (Figure S9b). Thus,  $[\text{Gd}(\text{OH})]^{2+}[\text{SN-38}]^{2-}$  IOH-NPs also show greenish emission at 500-650 nm ( $\lambda_{\text{max}} = 560$  nm) upon excitation at 400-500 nm, which is similar to pure SN-38 in alkaline solution as a reference.<sup>S3</sup>



**Figure S9.** Fluorescence spectra of DUT549-labelled  $[\text{Gd}(\text{OH})]^{2+}[\text{SN-38}]^{2-}$  IOH-NPs: a) with free DUT549 as a reference ( $\lambda_{\text{ex}} = 550$  nm,  $\lambda_{\text{em}} = 585$  nm), b) with SN-38 solution at pH = 8 as a reference ( $\lambda_{\text{ex}} = 475$  nm,  $\lambda_{\text{em}} = 560$  nm).

## References

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