Supplemental information:

Phosphonate Coating of Commercial Iron Oxide Nanoparticles for Nanowarming Cryopreserved Samples

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Experimental

CPA: VS55		
Component	**Chemical**	**Mol/L**
VS55	DMSO	3.1
	Formamide	3.1
	Propylene Glycol	2.2
	HEPES	0.01
Euro Collins Carrier solution	Glucose	0.19
	KH_2PO_4	0.01
	K_2HPO_4	0.04
	KCl	0.02
	$NaHCO_3$	0.01

Table S1. VS55 components. Chemicals and concentrations in the CPA VS55, with Euro Collins as the carrier solution.

NMR analysis

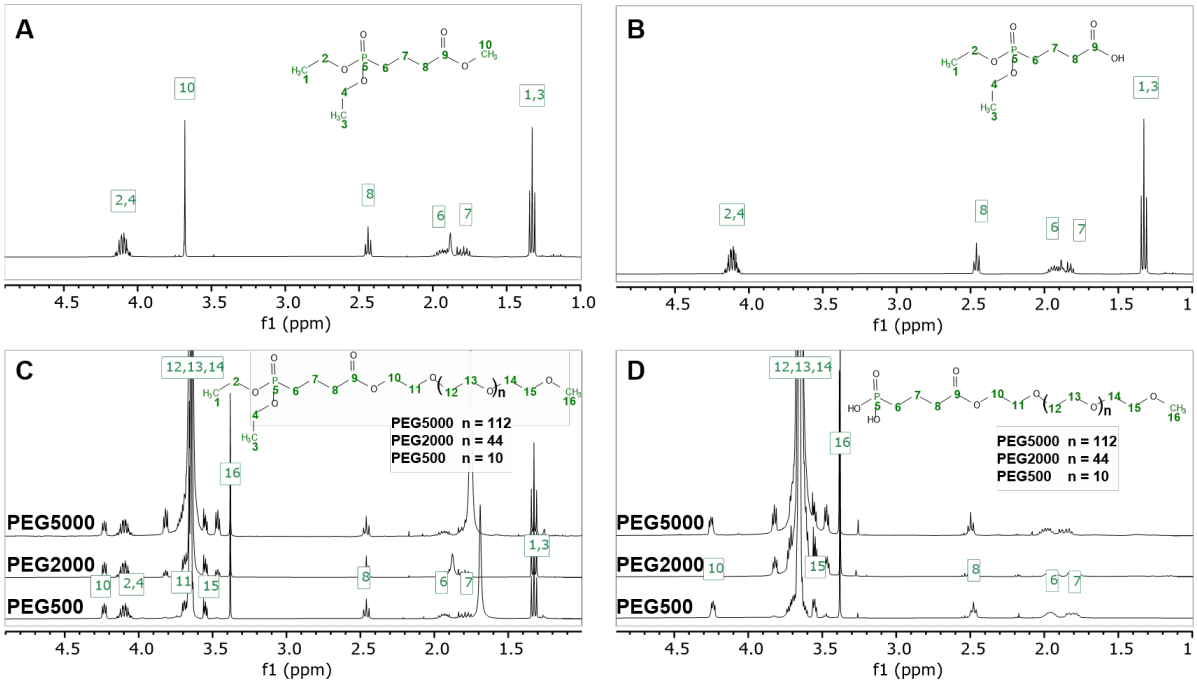

Fig S1. Area of interest ^{1}H NMR (400 MHz, $CDCl_3$) NMR spectra for synthesis **A.** Methyl 4-(diethoxyphosphoryl)butanoate (**2**); **B.** 4-(diethoxyphosphoryl)butanoic acid (**3**) **C.** PEG 4-(diethoxyphosphoryl)butanoate (**4**); **D.** (PEG-4-oxobutyl)phosphonic acid (**5**)

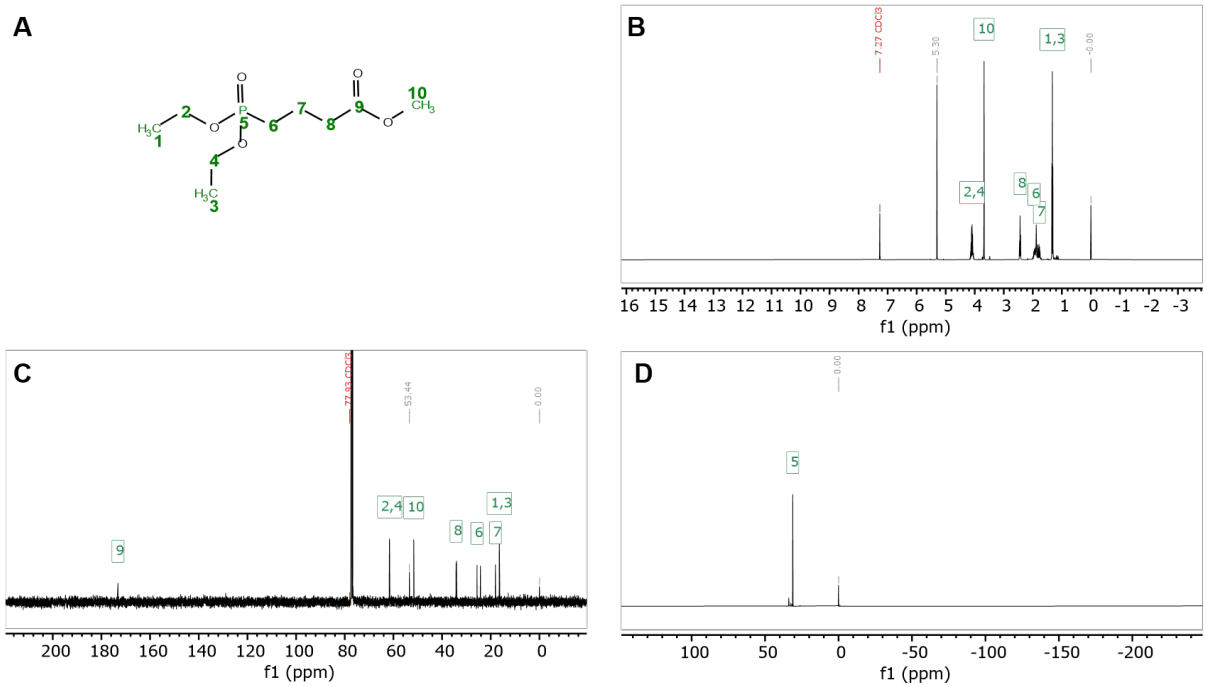

Fig S2. NMR spectra of methyl 4-(diethoxyphosphoryl)butanoate (**2**) **A.** Chemical structure **B.** ^{1}H NMR (400 MHz, $CDCl_3$) **C.** ^{13}C NMR (101 MHz, $CDCl_3$) **D.** ^{31}P NMR (162 MHz, $CDCl_3$)

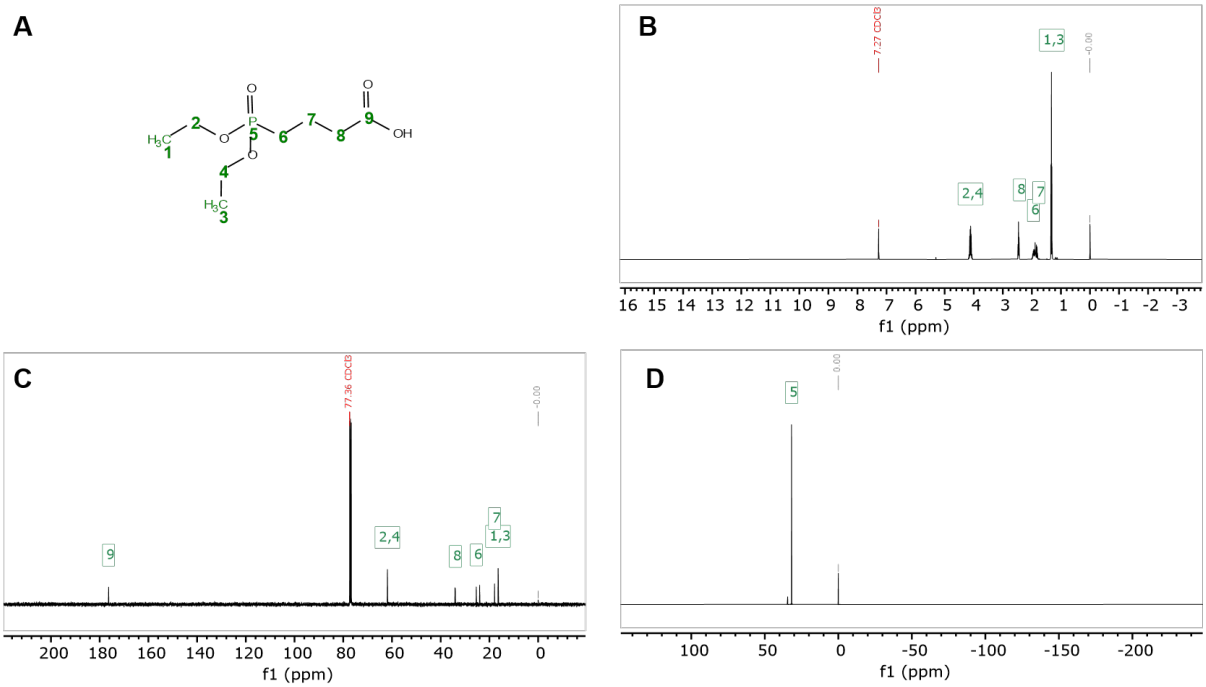

Fig S3. NMR spectra of 4-(diethoxyphosphoryl)butanoic acid) (**3**) **A.** Chemical structure **B.** ^{1}H NMR (400 MHz, $CDCl_3$) **C.** ^{13}C NMR (101 MHz, $CDCl_3$) **D.** ^{31}P NMR (162 MHz, $CDCl_3$)

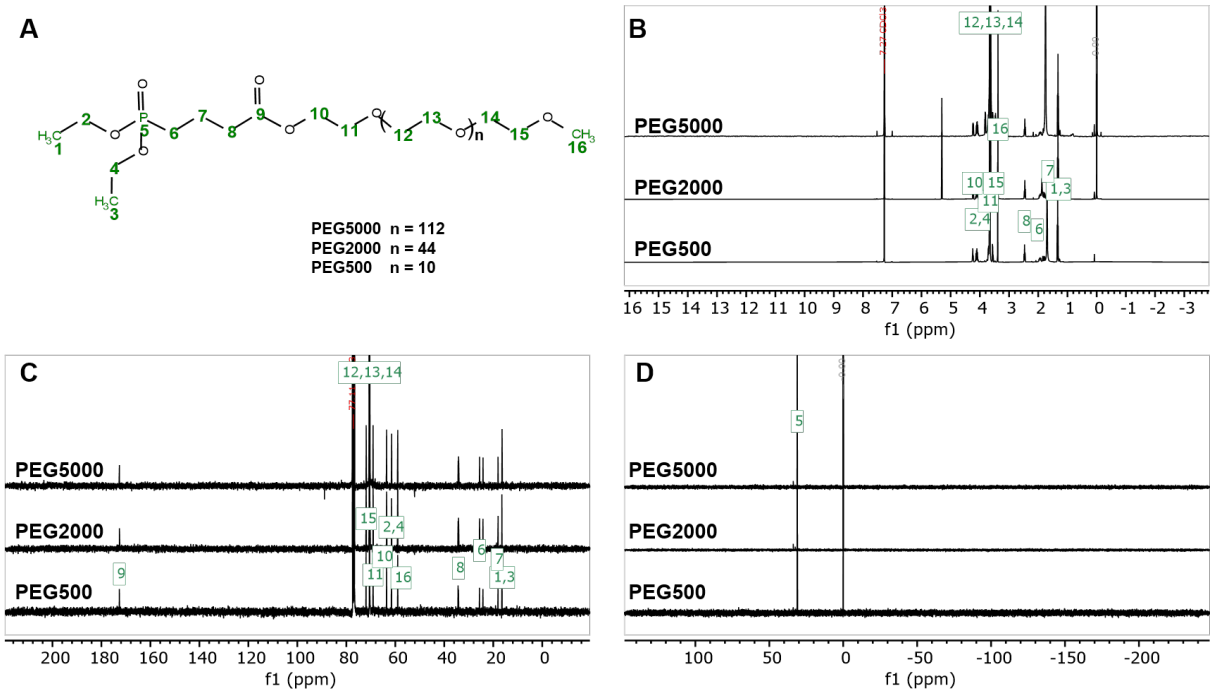

Fig S4. NMR spectra of PEG 4-(diethoxyphosphoryl)butanoate (**4**) **A.** Chemical structure **B.** ^{1}H NMR (400 MHz, $CDCl_3$) **C.** ^{13}C NMR (101 MHz, $CDCl_3$) **D.** ^{31}P NMR (162 MHz, $CDCl_3$)

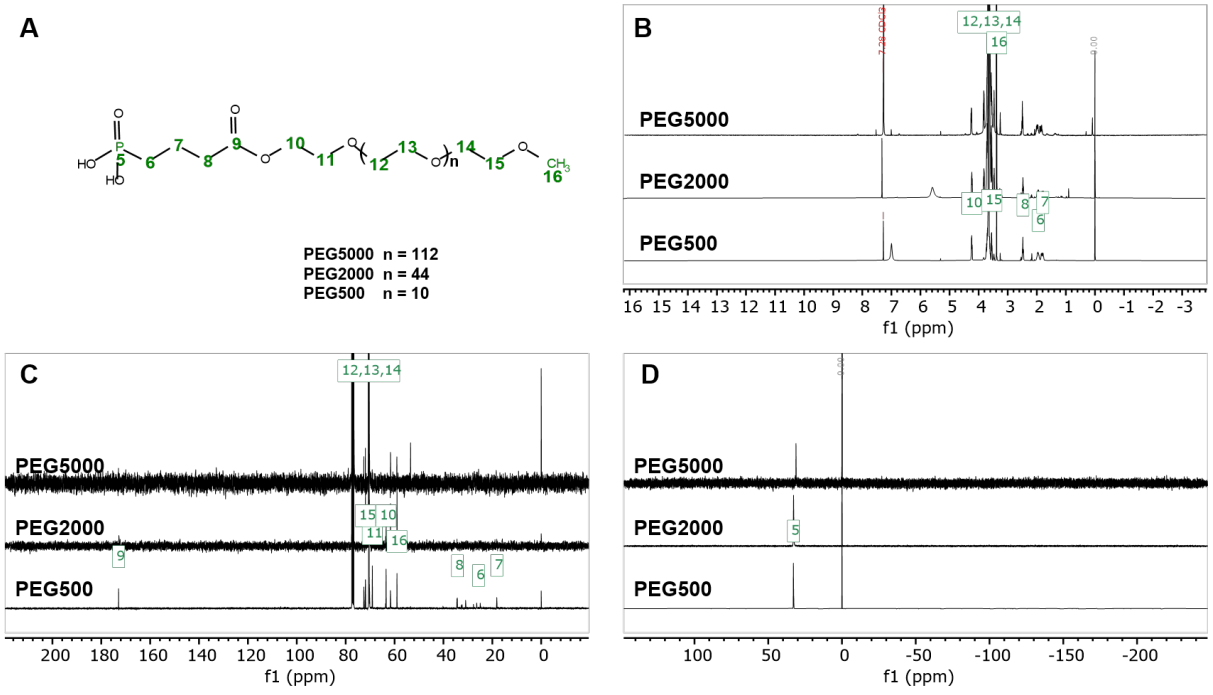

Fig S5. NMR spectra of (PEG-4-oxobutyl)phosphonic acid (**5**) **A.** Chemical structure **B.** ^{1}H NMR (400 MHz, $CDCl_3$) **C.** ^{13}C NMR (101 MHz, $CDCl_3$) **D.** ^{31}P NMR (162 MHz, $CDCl_3$)

IR data:

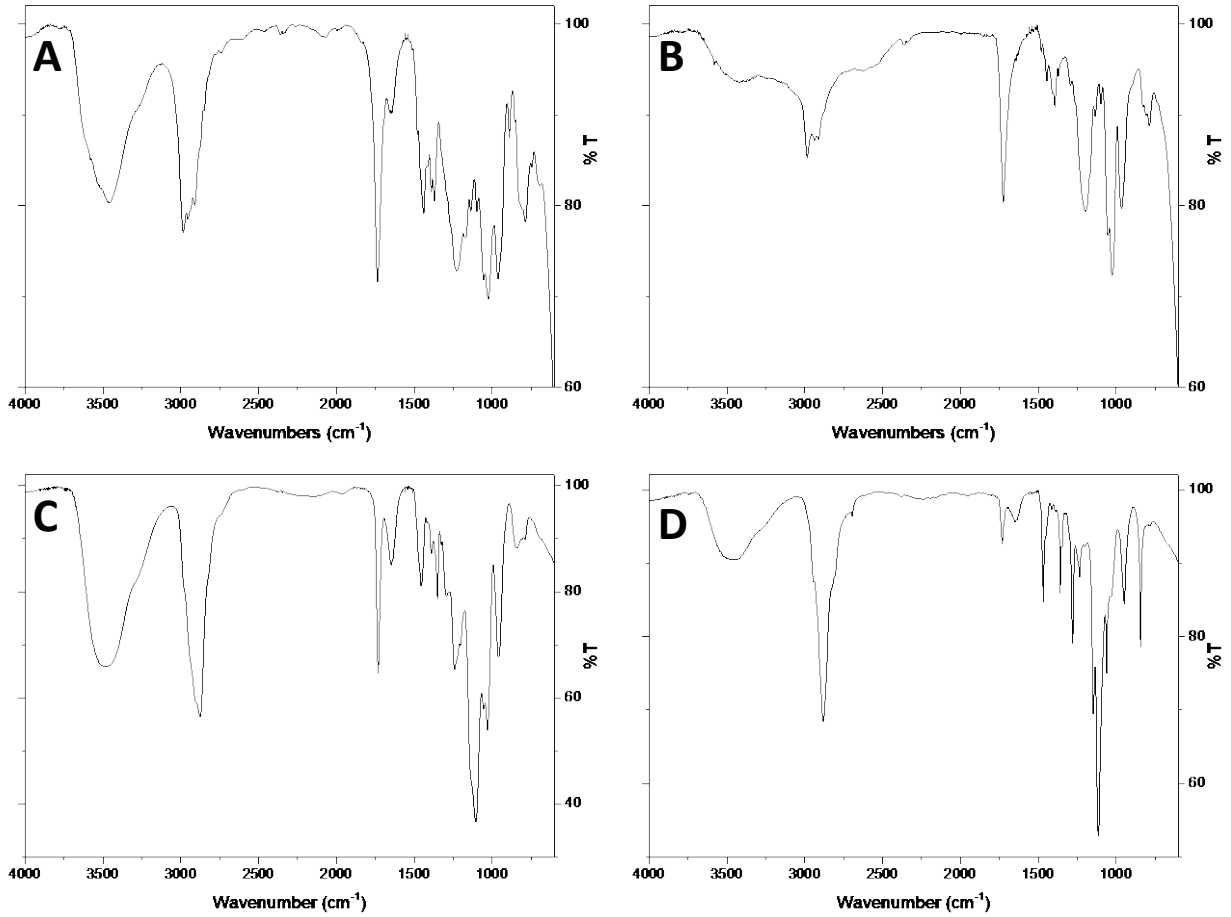

Figure S6. FT-IR spectra of (A) compound **2**, (B) compound **3**, (C) compound **4** PEG500, and (D) compound **4** PEG2000.

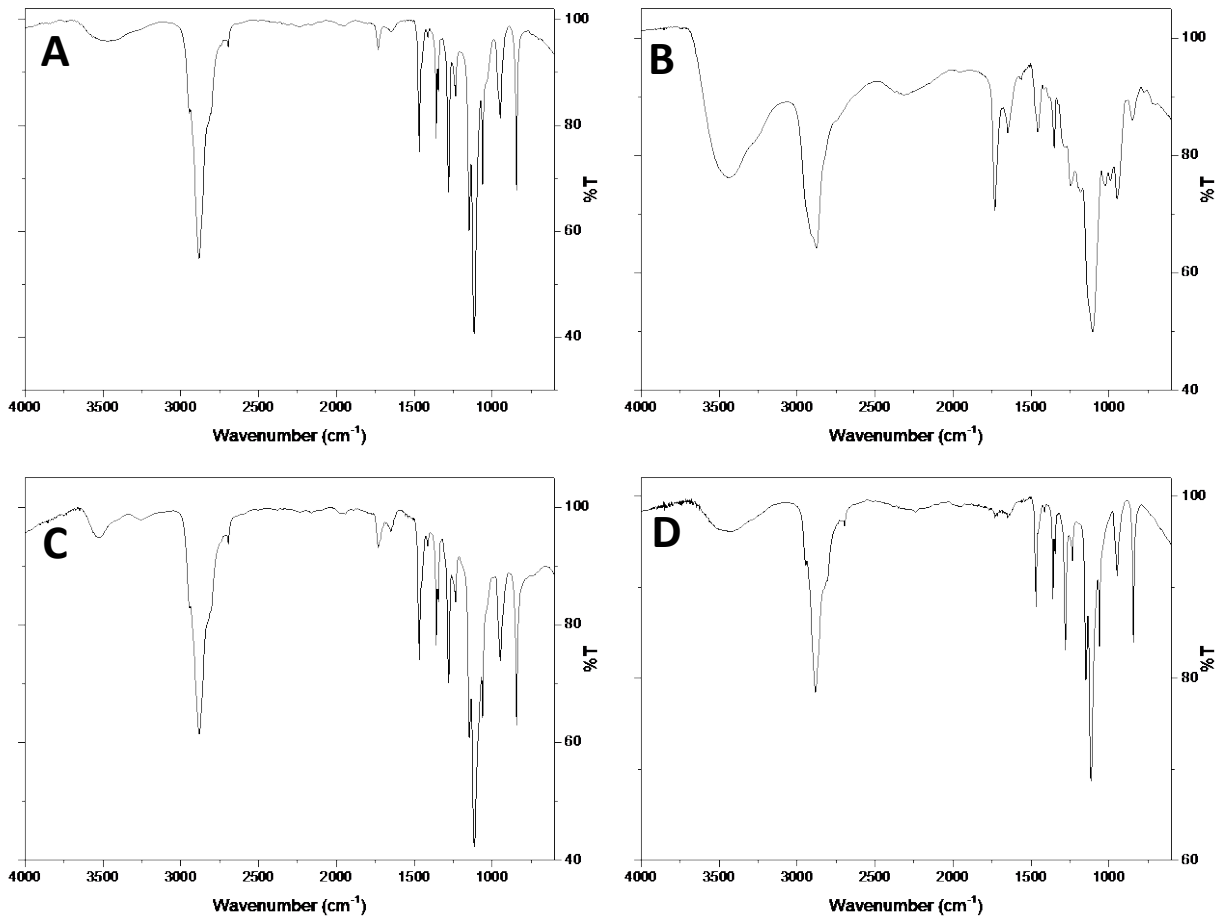

Figure S6B. FT-IR spectra of (A) compound **4** PEG5000, (B) compound **5** PEG500, (C) compound **5** PEG2000, and (D) compound **5** PEG5000.

MS data:

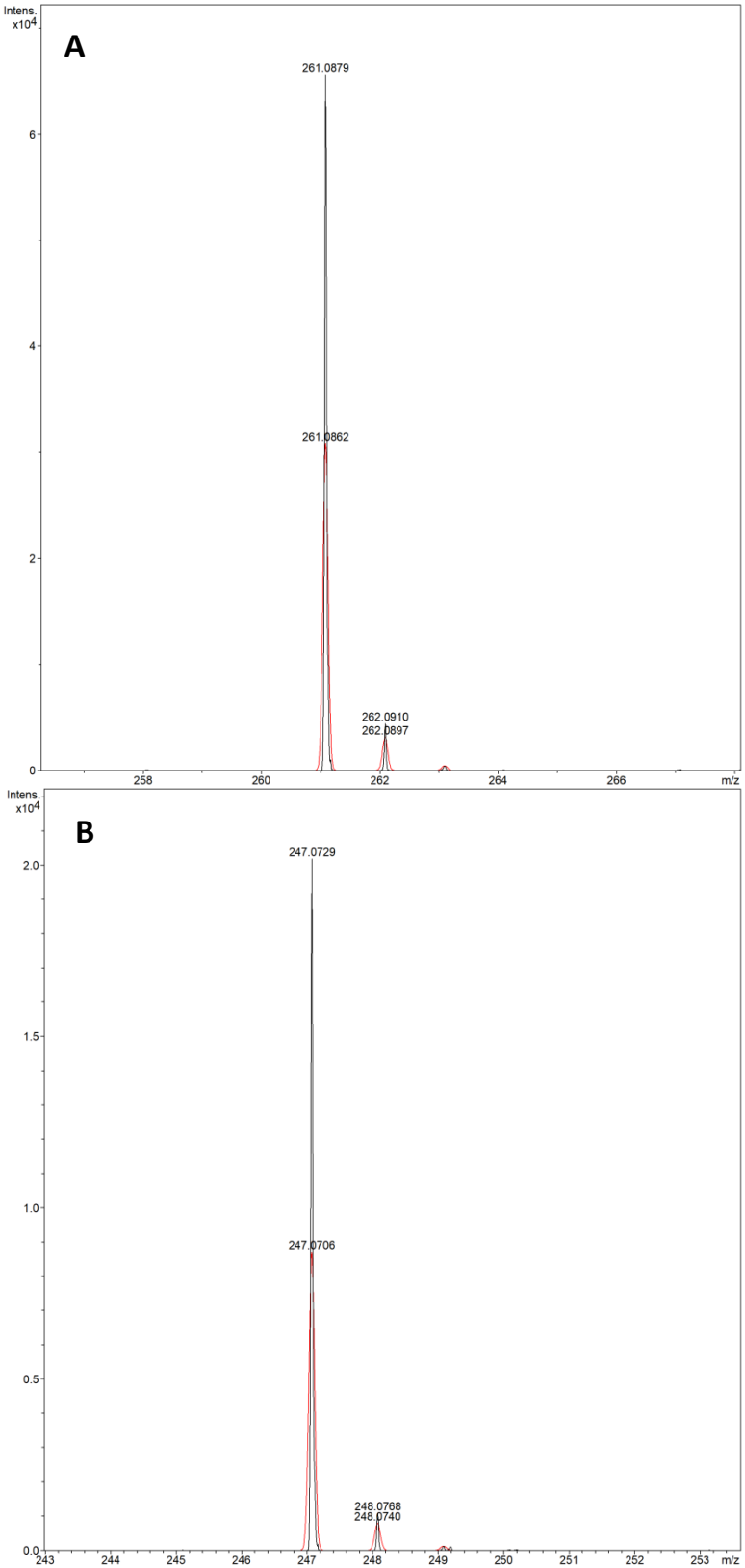

Figure S7. ESI-MS spectrum of (A) compound **2** ([M+Na]$^+$) and (B) compound **3** ([M+Na]$^+$), experimental (black) and calculated (red).

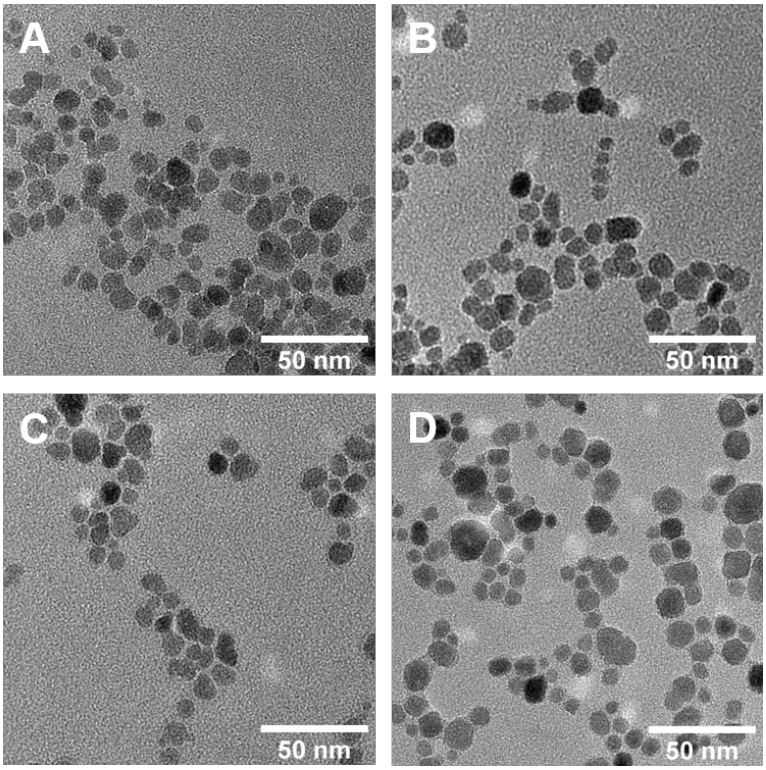

Figure S8. TEM images of IONPs. **A.** EMG1200-PEG5000, **B.** EMG308-PEG5000 **C.** EMG1200, **D.** EMG308

Reaction	RXN yield	Chemical	MW (g/mol)	Eqv. (moles)	Cost ($/g Fe IONP)	Labor for 100 g (hours)	
						Synthesis	Purification
a	70%	Methyl-4-bromobutyrate	181	1	$ 0.12	1	4
		Triethyl phosphite	166	1.5	$ 0.03		
b	99%	Sodium hydroxide	40	2	$ 0.01	0.25	3
		H2O	*18*	*139*	$ -		
c	95%	mPEG 5000	5000	1	$ 1.10	0.25	3
		DCC	206	1.1	$ 0.08		
		DMAP	122	0.01	$ 0.00		
		DCM	*85*	*156*	$ 0.48		
d/e	80%	TMSBr	153	4	$ 1.41	1	10
		DCM	*85*	*156*	$ 0.46		
		Isoprop	*60*	*5000*	$ 6.87		
f	-	**EMG 308**	-	-	**$ 102.00**	10.5	20
		MeOH	*32*	*619*	$ 0.73		
		H2O	*18*	*4167*	$ -		
				Total cost	**$ 113.29**		
f	-	**EMG1200**	-	-	**$ 31.25**	10.5	20
		MeOH	*32*	*619*	$ 0.73		
		DCM	*85*	*1565*	$ 3.66		
				Total cost	**$ 46.20**		

Table S2. Cost analysis of EMG1200 or EMG308 coating. Chemicals cost per g Fe of IONPs and the labor hours estimated for a large scale synthesis of 100 g of IONPs. Largest cost of materials comes from the cost of the IONP in either case. Costs are estimated from current available prices as of February 2022.

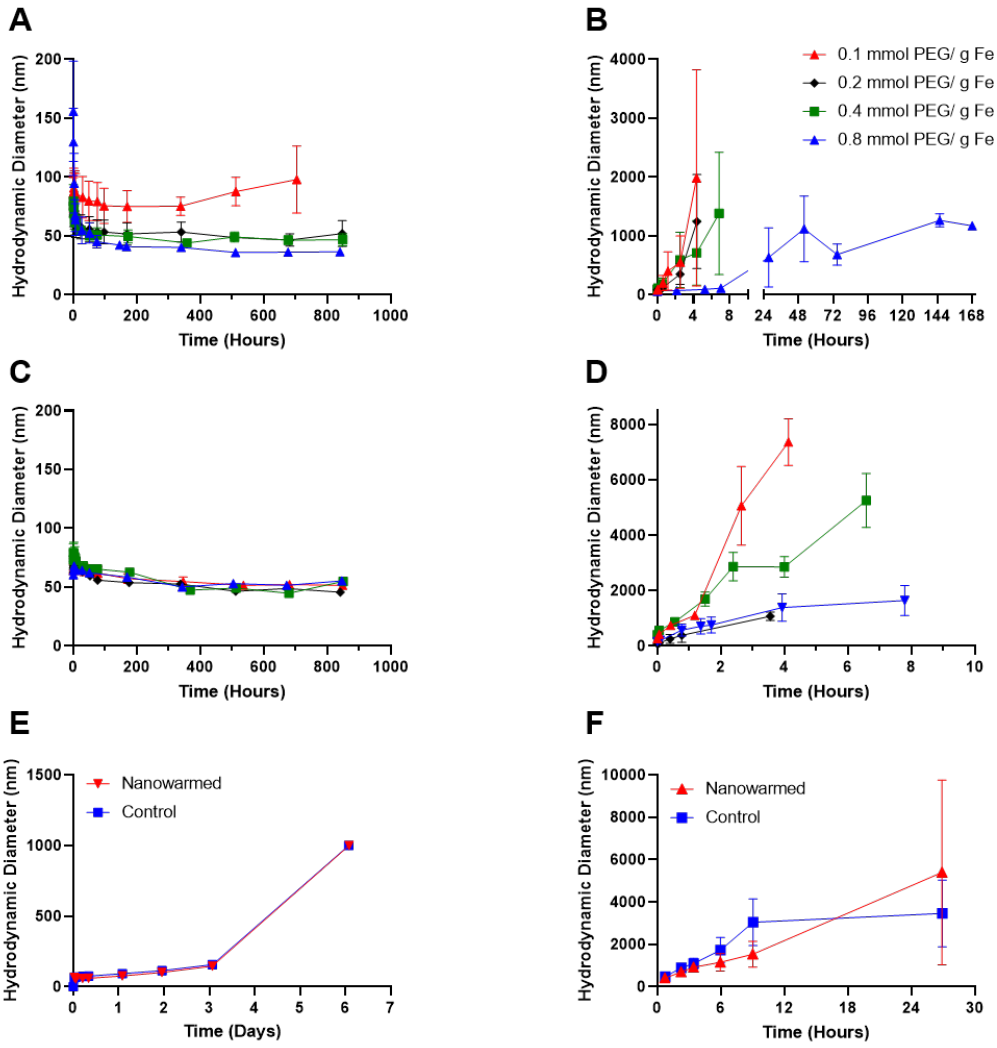

Fig S9. DLS stability of IONPs with increasing concentration of PEG5000 weighted by intensity. **A.** 1200-PEG5000 in H_2O, **B.** 1200-PEG5000 in VS55. **C.** 308-PEG5000 in H_2O, **D.** 308-PEG5000 in VS55. Upper left legend applies for all; Red triangle is 0.1 mmol PEG/g Fe, black square is 0.2 mmol PEG/g Fe, green triangle is 0.4 mmol PEG/g Fe, Blue diamond is 0.8 mmol PEG/g Fe. **E.** 1200-PEG5000 at 0.8 mmol PEG/g Fe with or without cryopreservation and nanowarming. **F.** 308-PEG5000 at 0.8 mmol PEG/g Fe with or without cryopreservation and nanowarming.

HRMAS sample preparation:

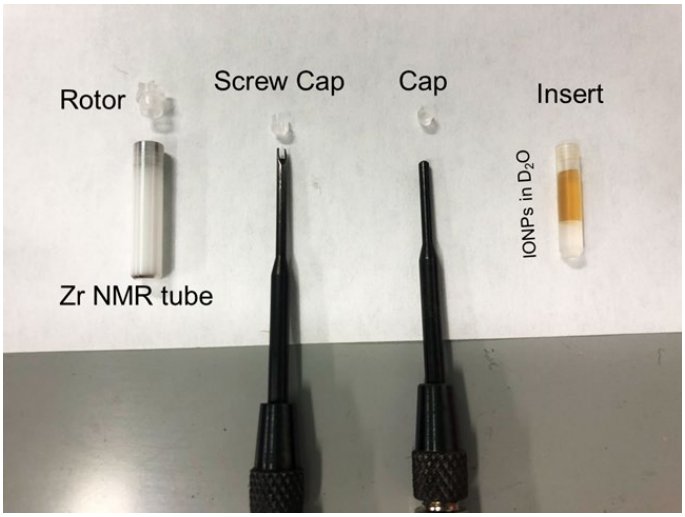

Figure S10. HRMAS NMR tube set up and solution color for reference

HRMAS NMR spectra of all coated samples:

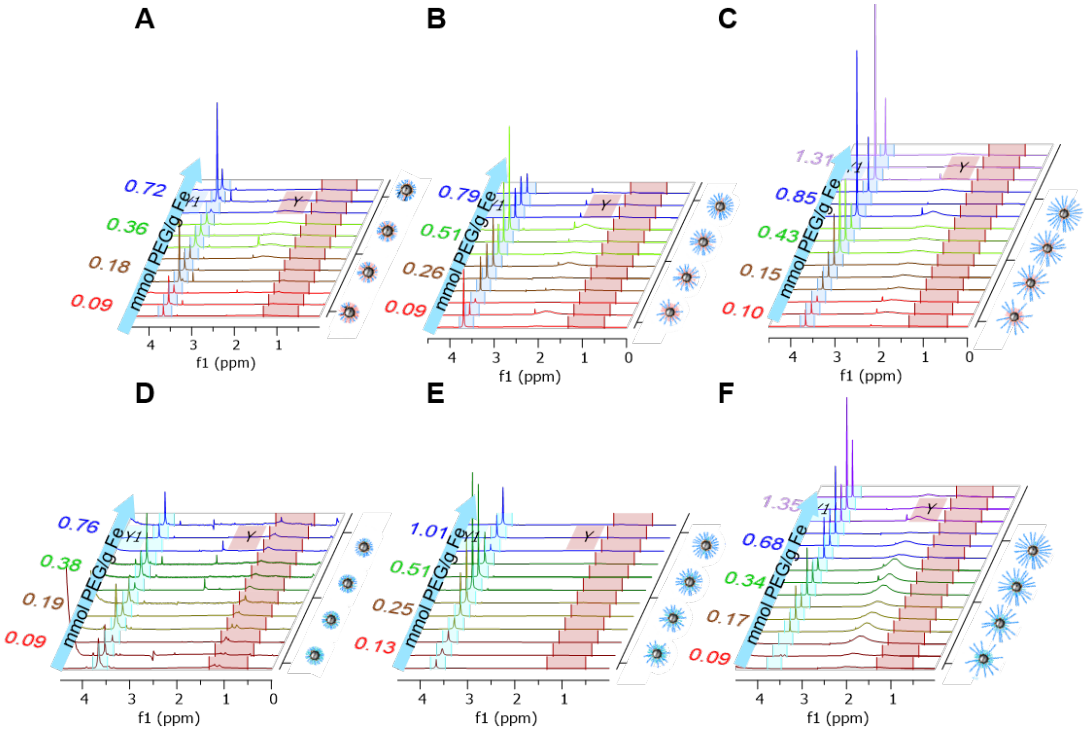

Fig S11. ^{1}H HRMAS NMR (600 MHz, D_2O) of coated PLink-PEG coated IONPs. **A.** EMG1200 coated with PEG500. **B.** EMG1200 coated with PEG2000. **C.** EMG1200 with PEG5000. **D.** EMG308 coated with PEG500. **E.** EMG308 coated with PEG2000. **F.** EMG308 coated with PEG5000.

The 0.5-1.32ppm fatty acid region is broad when using D_2O, but the region is more resolved when using DMSO (data not shown). Unfortunately, DMSO d-6 provided a larger H_2O peak (3.33 ppm) which overlaps with the PEG. Accurate integration of PEG when using DMSO d-6 as solvent is not possible due to peak, so the data is not included. Chloroform was also tried as a solvent, but shimming was not possible. A clear residual solvent peak must be present to shim. In chloroform the residual solvent peak is already so narrow shimming effects cannot be clearly discerned.

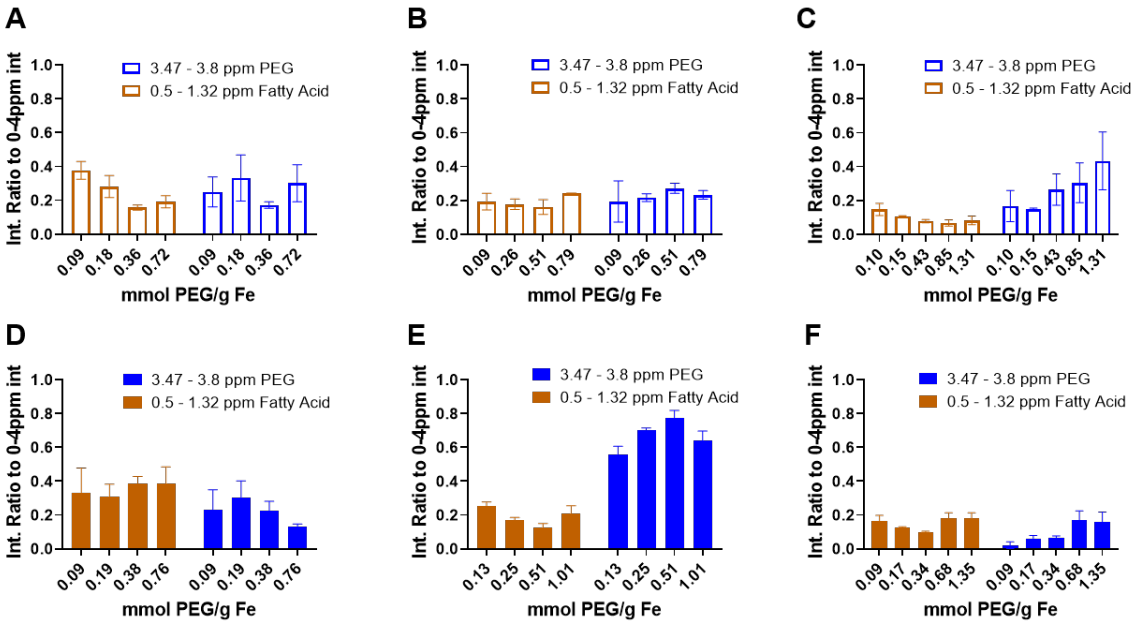

Figure S12. ^{1}H HRMAS NMR analysis of PEG peak to fatty acid peak of coated PLink-PEG coated IONPs. Integral of area from 0 to 4ppm was normalized to 1, integral from 3.47 to 3.8ppm is PEG (blue) and integral from 0.5 to 1.32 ppm is fatty acid (orange). Spectra was normalized to total area of interest (0-4ppm) to account for differences in resolution between samples. **A.** EMG1200 coated with PEG500. **B.** EMG1200 coated with PEG2000. **C.** EMG1200 with PEG5000. **D.** EMG308 coated with PEG500. **E.** EMG308 coated with PEG2000. **F.** EMG308 coated with PEG5000.

TGA of all PEG5000 coated samples

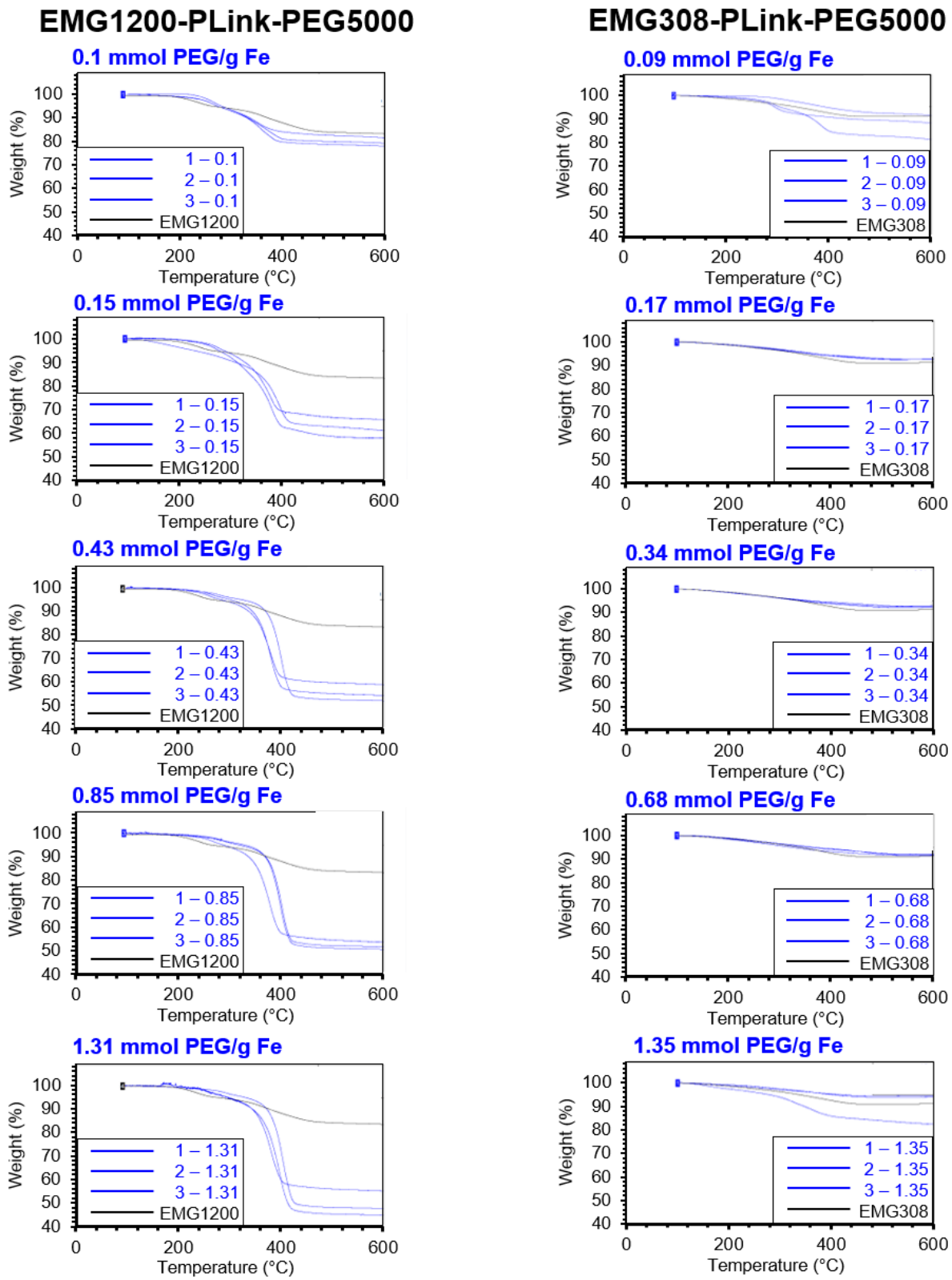

Fig S13. TGA data for PLink PEG5000 at various concentrations of PEG coated on IONPs. **Left**. EMG1200; uncoated – black, coated – blue. **Right**. EMG308; uncoated – black, coated – blue.

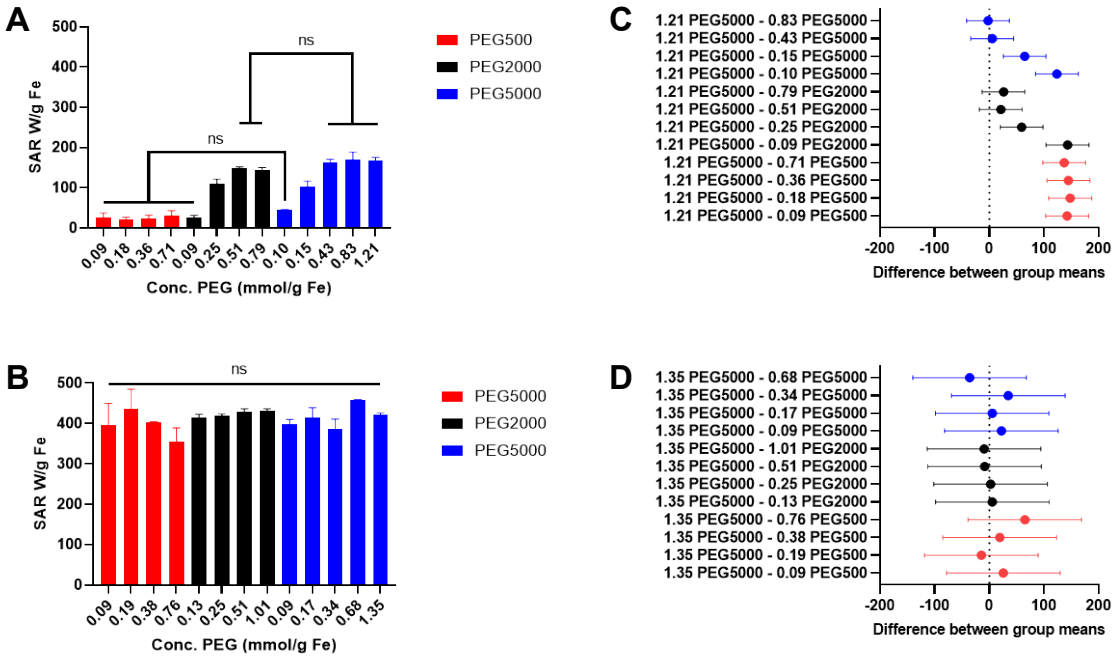

Fig S14. SAR in water statistical analysis. Statistical significance was determined by one-way ANOVA. ns denotes no significant difference: **A.** EMG1200 coated with PEG5000 and **B.** EMG308 coated with PEG5000. 95% Confidence Intervals Dunnett where statistically significant difference exists when the interval does not contain zero. **C.** EMG1200 coated with PEG5000 and **D.** EMG308 coated with PEG5000, confidence interval between two means of SAR values compared to a control of PEG5000 at maximum concentration.

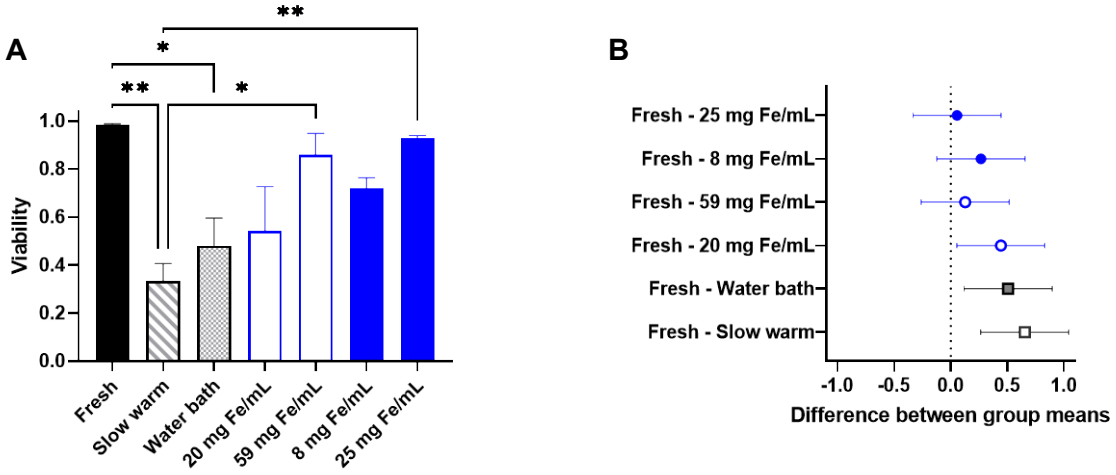

Fig S15. Cell cryopreservation rewarming statistical analysis **A**. Statistical significance was determined by one-way ANOVA comparison of all. Significance of P<0.05 denoted by *. Significance of P<0.01 denoted by **. **B** Confidence interval between two means of SAR values compared to a control of Fresh. 95% Confidence Intervals Dunnett where statistically significant difference exists when the interval does not contain zero.