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[Supplementary material]

Quantifying Biolipid (Rhamnolipid) Effects on the Aggregation Behavior of Engineered Nanoparticles

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Table S1: Optimized Ligand concentration based on yield (%) of Fe transferred, hydrodynamic diameter(nm), number of surfactant molecules/IONP.

Ligand	Optimized Concentration(mM)	Optimized Sonication time (min)	Yield of Fe transferred	Number mean Hydrodynamic diameter (nm	No. of ligands/IONP
CTAB	5	4	74.48%	14.4 ± 3.08	718±72
SDBS	10	6	88.23%	15.66 ± 2.8	830 ± 100

Table S2: Adsorption isotherm parameters of MonoRL and DiRL adsorbed on SDBS and CTAB-IONP.

Nanoparticle	Glycolipid	Langmuir	Freundlich	Redlich-Peterson	Sips
CTAB-IONP	**MonoRL**	Q_{max} = 7073.7	K_F =261.94	K_R = 171.94	K_S = 1.4799
		K_L = 0.05	n = 1.3	β = 2.39	β_S = 3.026
		R^2 = 0.5	R^2 = 0.78	α = 0.000035	α_S = 0.000227
				R^2 = 0.75	R^2 = 0.996
	DiRL	Q_{max} = 1944	K_F =260.96	K_R = 146.53	K_S = 0.129
		K_L = 0.098	n = 2.05	β = 1.577	β_S = 6.63
		R^2 = 0.59	R^2 = 0.56	α = 0.0087	α_S = 0.00009
				R^2 = 0.63	R^2 = 0.99
SDBS-IONP	**MonoRL**	Q_{max} = 454847	K_F = 12.6	K_R = 107.7	K_S = 6.56
		K_L = 0.00024	n = 0.64	β = 2.34	β_S = 1.76
		R^2 = 0.74	R^2 = 0.997	α = 8.9E-09	α_S = 0.00018
				R^2 = 0.74	R^2 = 0.995
	DiRL	Q_{max} = 396936	K_F =0.00009	K_R = 85.84	K_S = 3.39
		K_L = 0.0002	n = 0.187	β = 0.0001	β_S = 2.016
		R^2 = 0.48	R^2 = 0.91	α = 0.00001	α_S = 0.00001
				R^2 = 0.48	R^2 = 0.58

†**Langmuir isotherm (equation 1)**: It assumes homogeneous adsorption such that all sites possess equal affinity for the adsorbate. No adsorption takes place at a site on which the adsorbate is already adsorbed. where Q_e is the amount of adsorbate adsorbed (mg/g); $Q_{max}$is the maximum amount adsorbed,K_L is the Langmuir equilibrium constant and C_e is the residual/equilibrium concentration of the RLs.

$$Q_e = \frac{Q_{max} K_L C_e}{1 + K_L C_e} \quad (1)$$

Freundlich isotherm (equation 2): It assumes that the solute adsorption occurs on the adsorbent by multilayer adsorption and the adsorbent surface is composed of heterogeneous sites. where, K_F and n are the Freundlich adsorption constants related to sorption capacity and sorption intensity, respectively.

$$Q_e = K_F C_e^{1/n} \quad (2)$$

Redlich Peterson (equation 3): This model combines features of both Langmuir and Freundlich isotherms and is a three parameters multilayer sorption model. At low concentrations, it approaches Langmuir adsorption isotherm (as the β value tends to one) and at high concentration it approaches Freundlich adsorption isotherm. Here, K_r ,α and β are Redlich-Peterson isotherm constants.

$$Q_e = \frac{K_r C_e}{1 + \alpha C_e^{\beta}} \quad (3)$$

Sips (equation 4): Like Redlich Peterson, this is a three-parameter multilayer model. At low adsorbate concentrations, this model reduces to Freundlich isotherm, while at high concentrations, it predicts a monolayer adsorption capacity characteristic of the Langmuir isotherm. Here, K_s, $\alpha_{s,}$ β_s are Sips constant.

$$Q_e = \frac{K_s C_e^{\beta_s}}{1 + \alpha_s C_e^{\beta_s}} \quad (4)$$

Table S3: Grafting density of MonoRL and DiRL on CTAB-IONP.

		No. of RLs/IONP	**No. of RLs per CTAB grafted on IONP (2^{nd} layer has been calculated as- No. of RLs in second layer/No. of RLs in first layer)**
MonoRL	First layer	1325 ± 178	1.85 ± 0.24
	Second layer	1432 ± 294	1.08 ± 0.41
DiRL	First layer	829 ± 54.3	1.15 ± 0.08
	Second layer	681 ± 207	0.82 ± .29

As proposed in model Figure 4, the first layer of rhamnolipid is assumed to be formed just before the nanoparticles aggregates and becomes hydrophobic as shown in Fig 3 (12.5mg OC/l of DiRL and 20mg OC/l of MonoRL) and the subsequent second layer is where the IONP becomes stable again (50mg OC/l of MonoRL and DiRL is assumed). Each IONP is surrounded by 718 ± 72 CTAB ligands as shown in the SI and is used to calculate the number of rhamnolipid molecules attached per CTAB ligand for the first monolayer. The second layer is assumed to be formed on the already formed rhamnolipid monolayer.

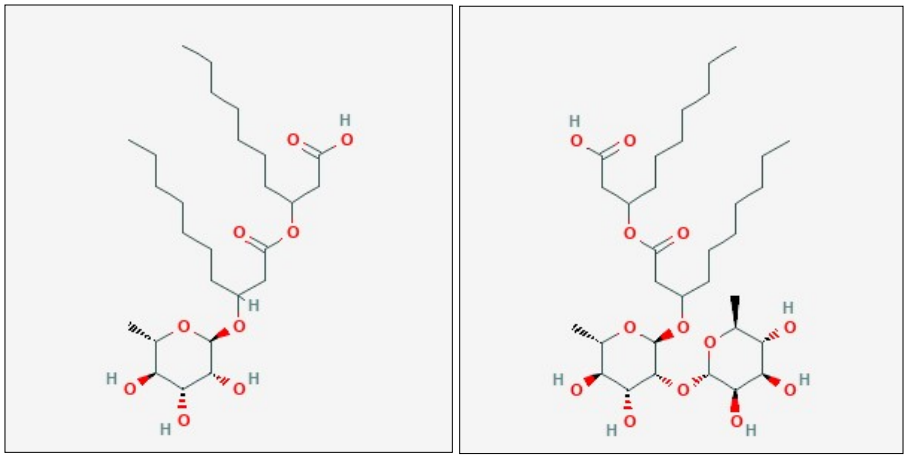


Figure S1. Chemical structure of MonoRL on the left and DiRL on the right.[1,2]

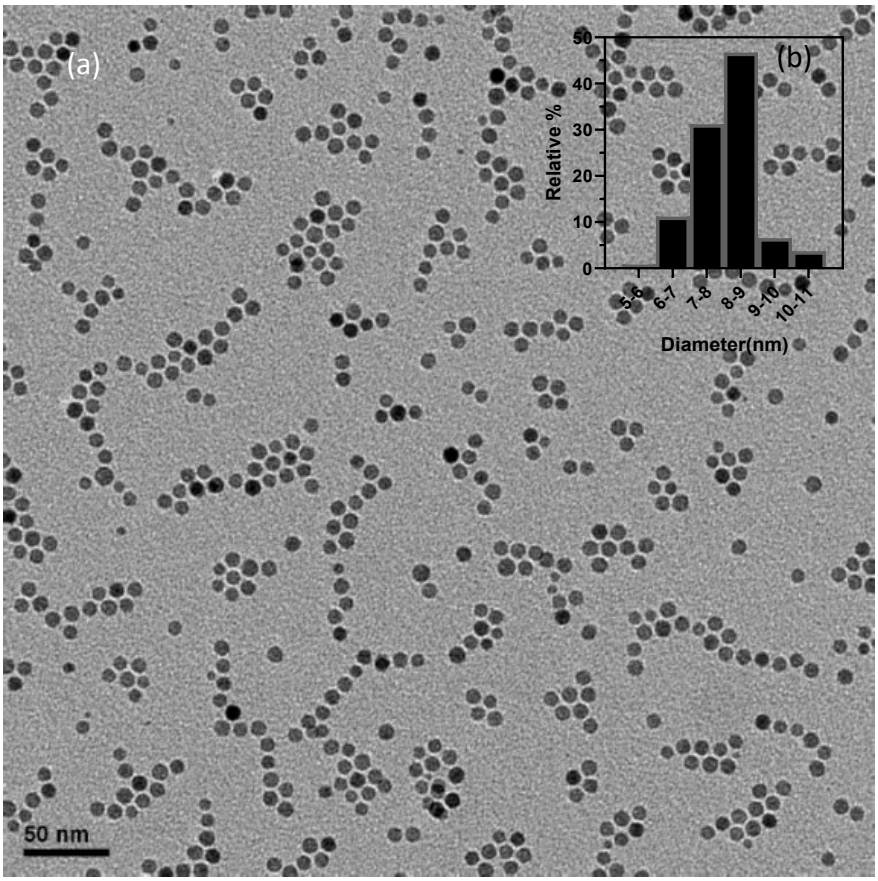


Figure S2: TEM image for (a) Oleic acid coated nanoparticles in hexane and (b) Relative% Vs Diameter in nm analyzed by using ImageJ software.

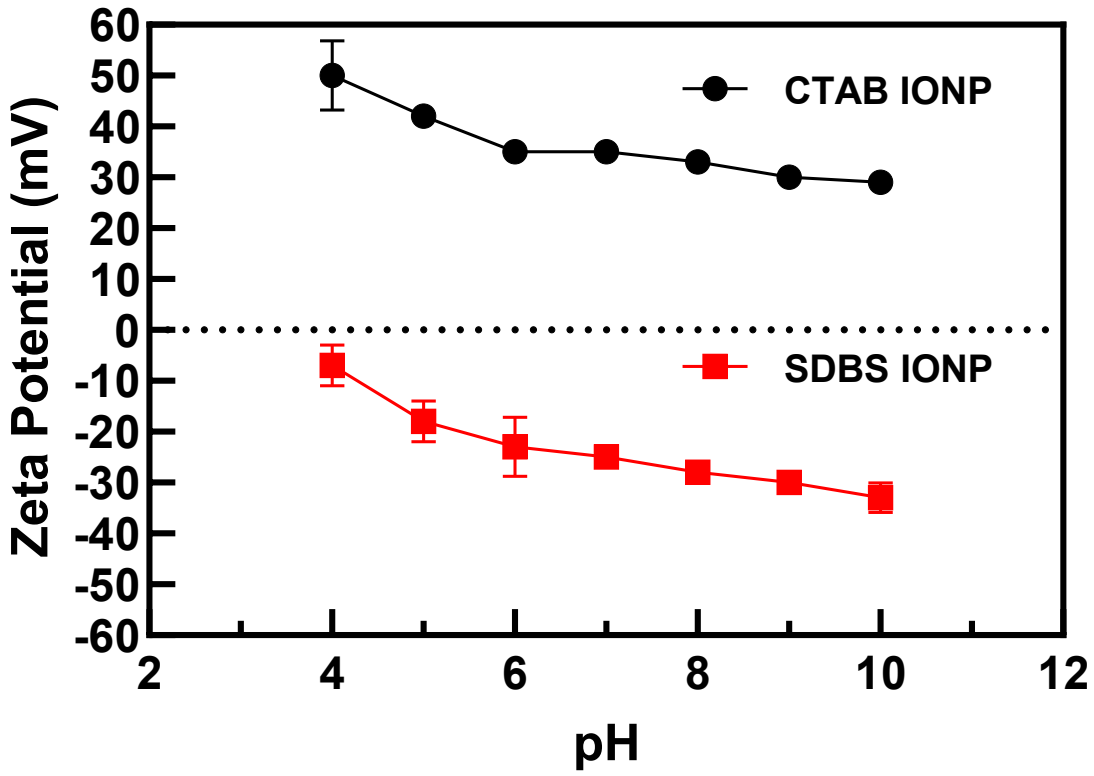


Figure S3: Zeta potential (mV) as a function of pH for CTAB-IONP and SDBS-IONP.

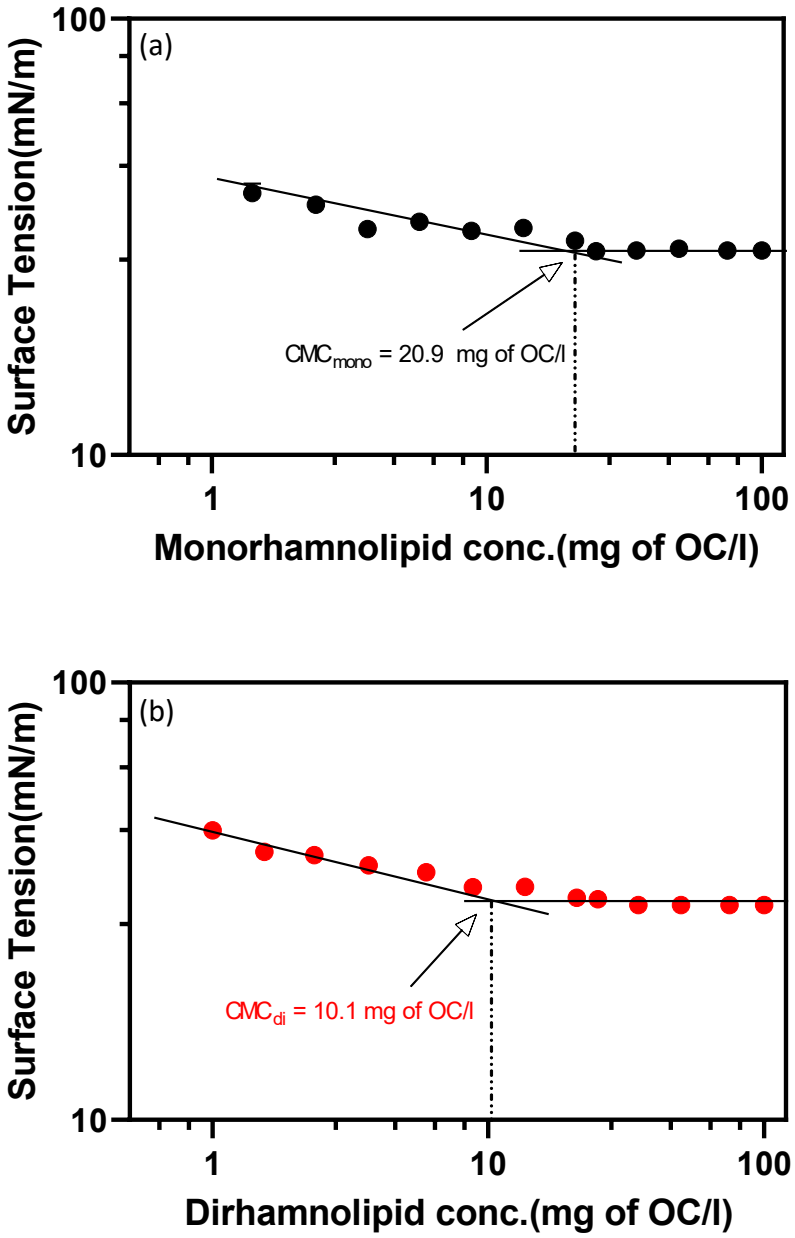
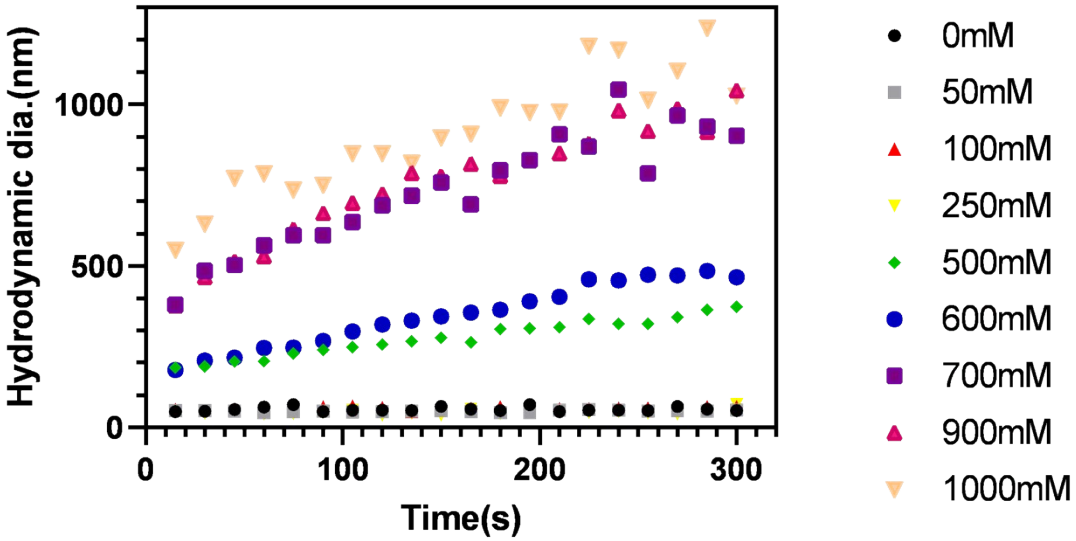


Figure S4: Log-log plot of surface tension Vs (a) MonoRL and (b) DiRL concentration. The CMC value of MonoRL and DiRL are 20.9 and 10.1mg of OC/l respectively.



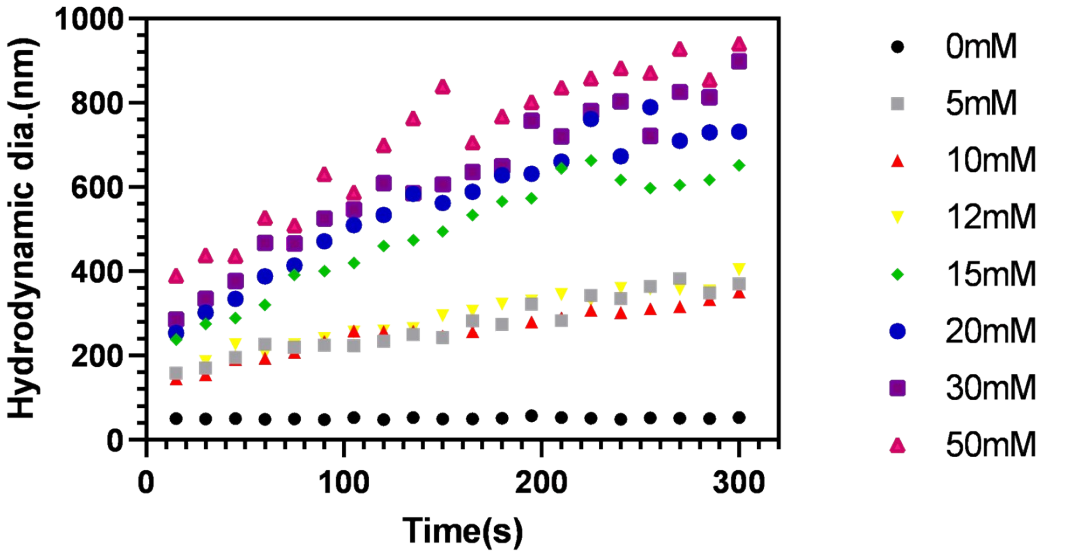


Figure S5: Intensity mean hydrodynamic diameter change with time for SDBS-IONP. (a) on addition of NaCl and (b)$MgCl_2$.700mM and 15mM of NaCl and $MgCl_2$ is the CCC value after which the IONPs undergo diffusion limited aggregation.

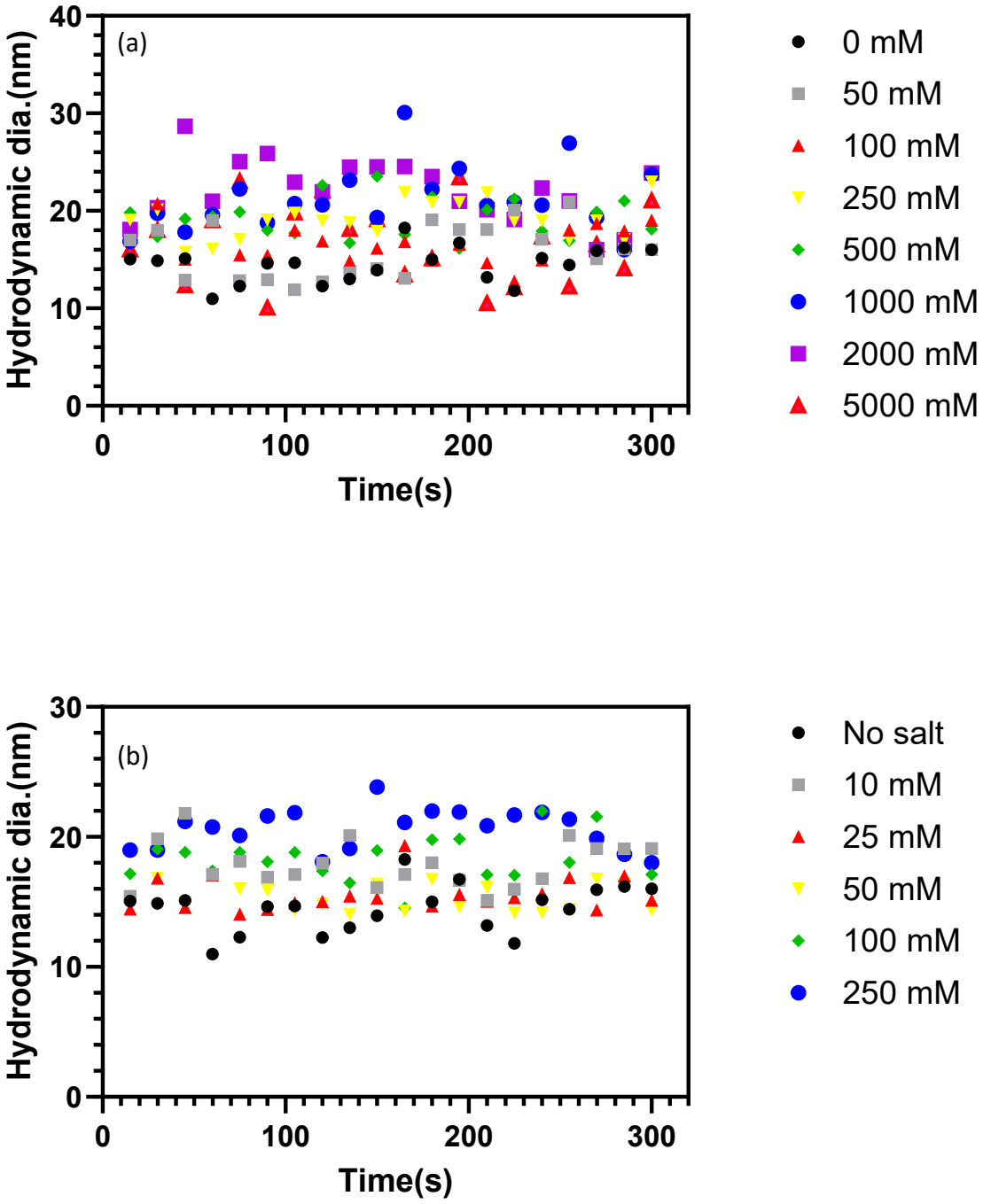


Figure S6: Number mean hydrodynamic diameter change with time for CTAB-IONP. (a) on addition of NaCl and (b)Na_2SO_4.

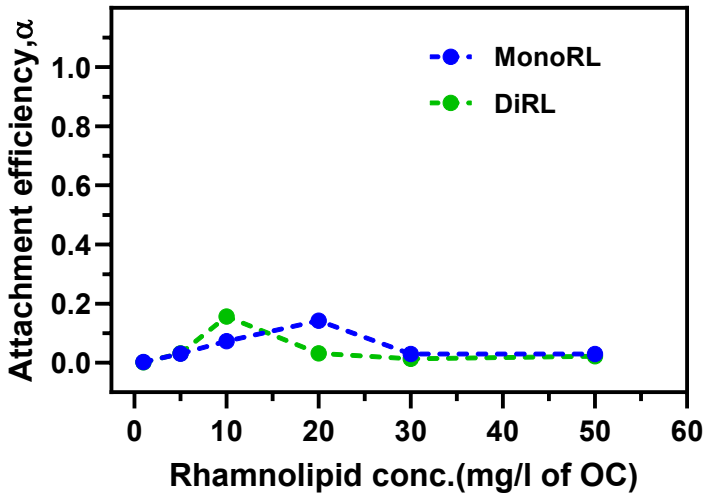


Figure S7: Attachment efficiency of 10mg/l of SDBS-IONP as a function of Rhamnolipid concentration.

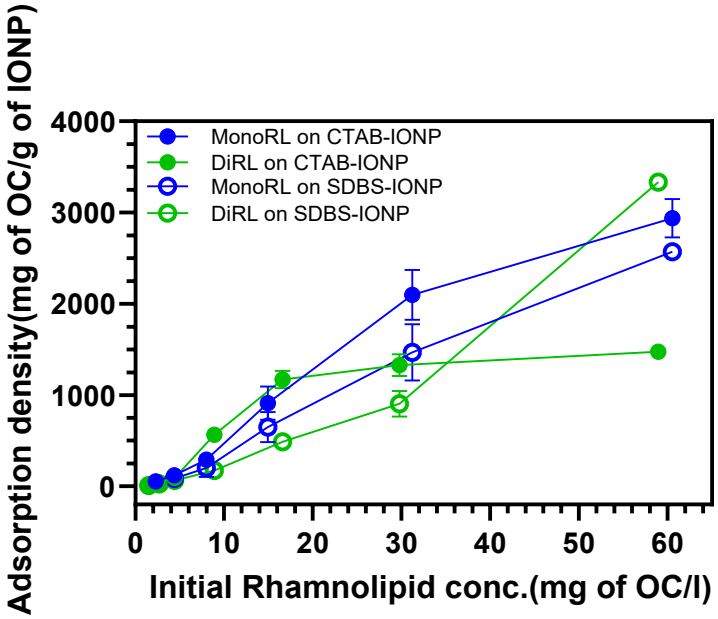


Figure S8: Adsorption density Vs initial conc. of Rhamnolipids.

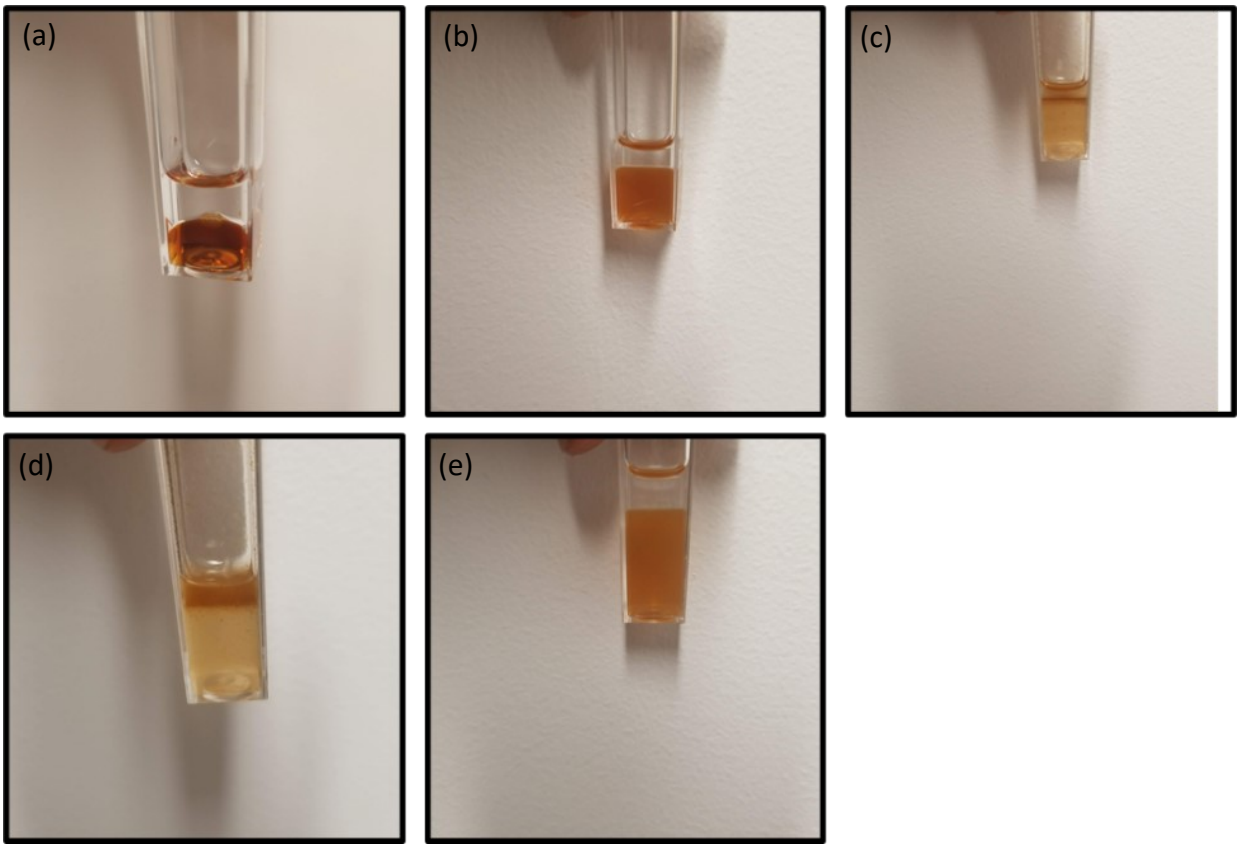


Figure S9: Partitioning of CTAB-IONP in hexane in presence of DiRL. (a) No DiRL (b) 0.5:1 (c) 1:1 (d)1.5:1, and (e)3:1 ratio of DiRL to CTAB-IONP. The upper layer is that of hexane and addition of rhamnolipid at point (c) and (d) causes partitioning of CTAB-IONPs to hexane phase. At highest concentrations of DiRL there is no partitioning visible.

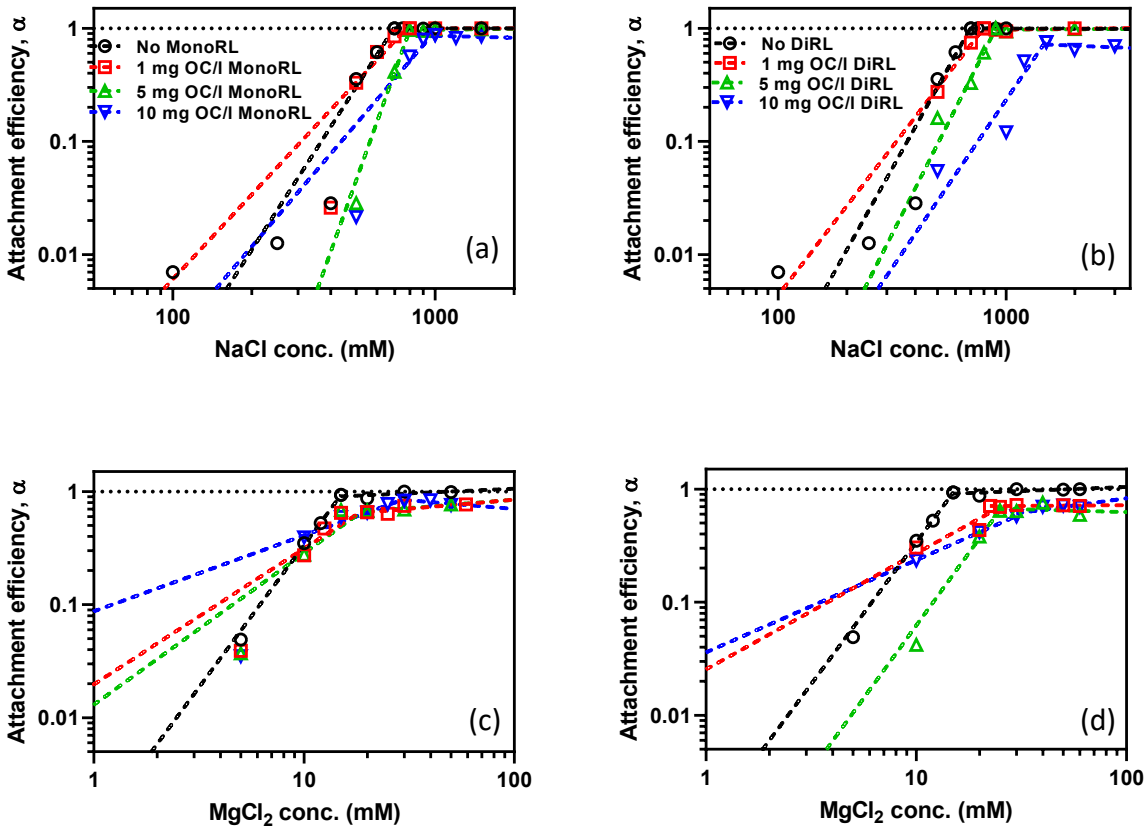


Figure S10: Attachment efficiency of SDBS-IONPs at PH 7.2 in presence of (a) MonoRL and NaCl, (b) DiRL and NaCl, (c) MonoRL and $MgCl_2$ and (d) DiRL and $MgCl_2$ where the black hollow rounds, red hollow square, green hollow triangle and blue inverted hollow triangle shows the no rhamnolipids, 1,5,10mg/l of OC respectively

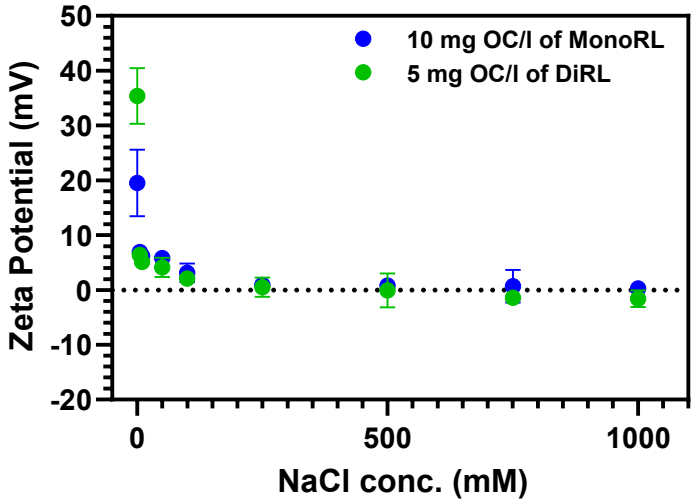


Figure S11: Zeta potential vs NaCl concentration (mM) In Regime 1 for points A of monoRL (10 mg/L of OC) and diRL (5 mg/L of OC).

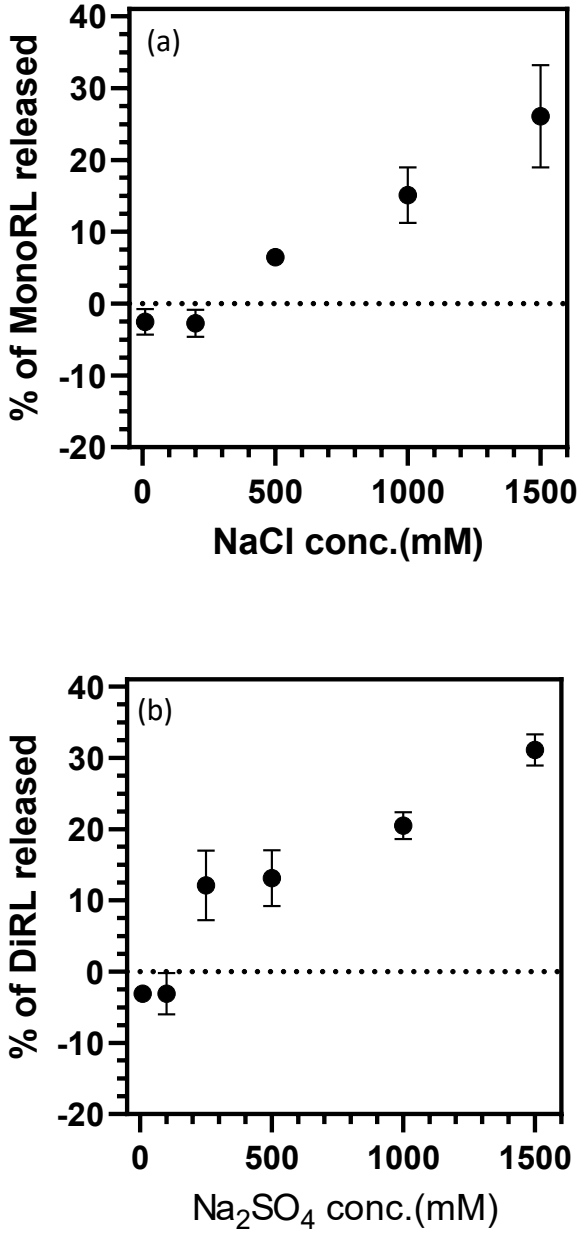


Figure S12: Percentage of (a) MonoRL and (b) DiRL released from CTAB-IONP on addition of NaCl.

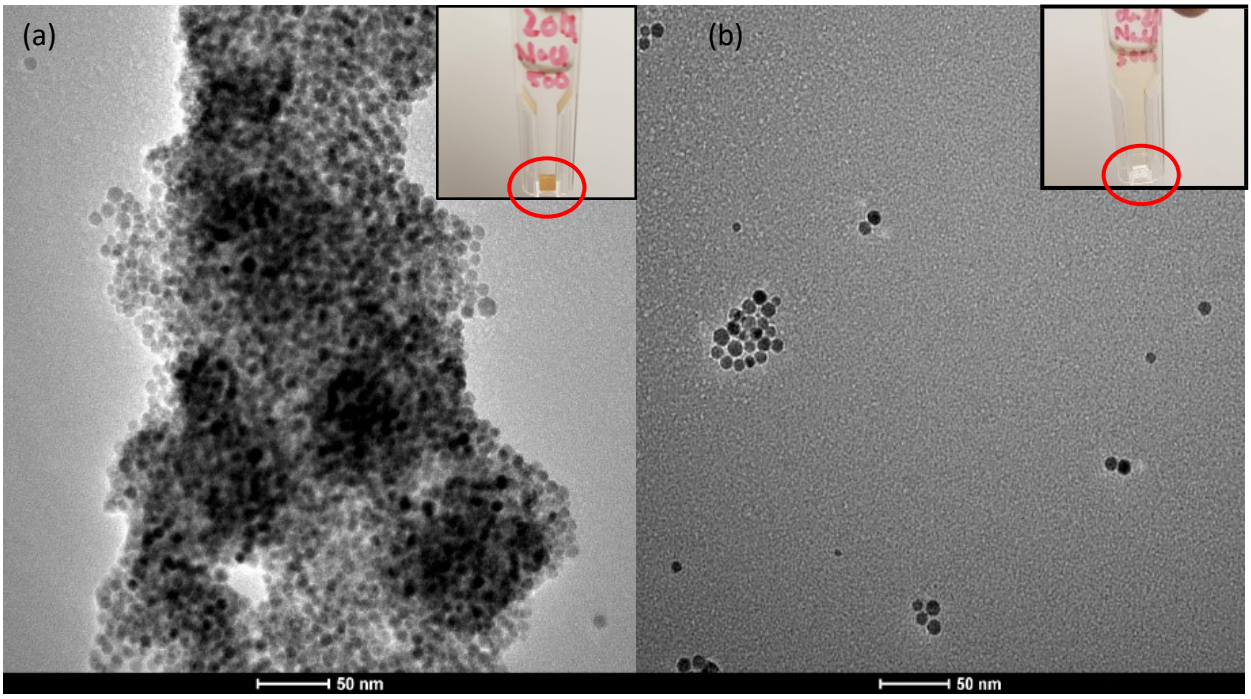
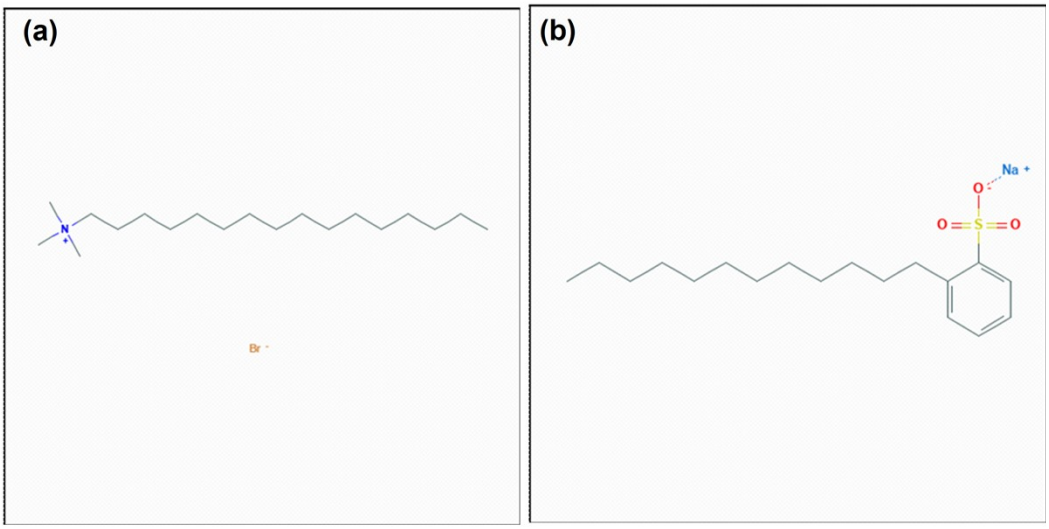


Figure S13: Representative TEM image of CTAB-IONP in presence of 20mg/l of OC MonoRL at 500mM NaCl conc. in the left and 2000mM in the right collected after 1 h after the start of reaction. Macroscopic view of the samples are attached at the top right which were collected after 12 h.



Grafting density calculation

Figure S14: Structure of CTAB and SDBS ligands are depicted in Fig(a) and (b) respectively

‡Total CTAB attached to IONPs (analyzed by TOC analyzer) = 178.21 mg/L as OC

Total SDBS attached to IONPs = 256.57 mg/l as OC

The bilayer consists of oleic acid + CTAB/SDBS

The total number of carbon atoms in one entity of CTAB-IONP =18C (oleic acid) + 19C(CTAB) = 37C

The total number of carbon atoms in one entity of SDBS-IONP= 18C (oleic acid) + 18C(SDBS) =36C

Density of IONPs as magnetite = 5g/cm^3

Assuming the diameter of 8 nm,

Volume of one IONP = $\frac{4}{3}\pi r^3$ = 2.68 *10^{-25} m^3

No. of IONPs in the solution = $\frac{Mass\ of\ Fe\ from\ ICP-OES}{\frac{Density\ of\ IONP\ as\ magnetite}{Volume\ of\ one\ IONP}}$

No. of IONPs in the CTAB-IONP solution = 3.36*10^{17}

No. of IONPs in the SDBS-IONP solution = $4.31*10^{17}$

Concentration of CTAB in the suspension= 178.21/ (37*12*1000) = 0.000401M

Concentration of SDBS in the suspension= 256.57/ (36*12*1000) = 0.000594M

1 mole contains $6.022 * 10^{23}$ entities, hence total number of CTAB entities in suspension = $2.41*10^{20}$

Total number of SDBS entities in suspension = $3.57*10^{20}$

Number of CTAB ligand attached to IONP = 718.2 per IONP

Number of SDBS ligand attached to IONP = 830.5 per IONP

References

1 Rhamnolipid 1 | C32H58O13 - PubChem https://pubchem.ncbi.nlm.nih.gov/compound/5458394 (accessed Jun 16, 2020).

2 Rphdhd | C26H48O9 - PubChem https://pubchem.ncbi.nlm.nih.gov/compound/162246 (accessed Jun 16, 2020).