SUPPLEMENTARY INFORMATION

Metal-enzyme frameworks: Role of metal ions in promoting enzyme self-assembly on α-zirconium (IV) phosphate nanoplates

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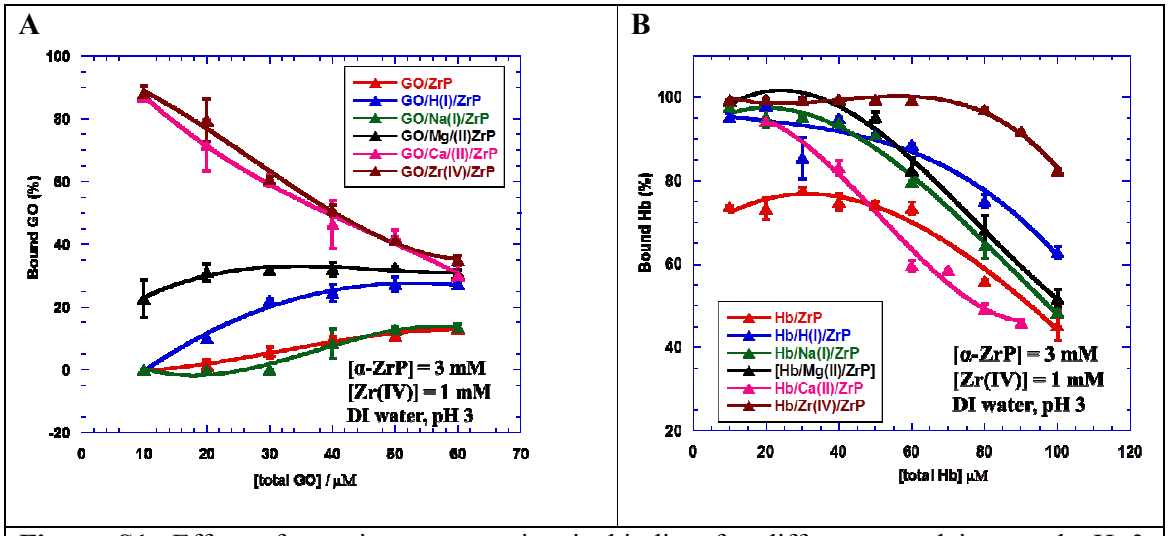

Figure S1*:* Effect of protein concentration in binding for different metal ions and pH *3*. Extent of binding against protein concentration in the presence of xpecific metal ions. **A.** GO/metal/α-ZrP data shows that Zr(IV) increased the affinity of GO the largest, when compared to any other system. **B**. Hb/metal/α-ZrP binding data which shows that after certain concentrations lead to maximum loading. Studies done with 1 mM metal concentration in DI water. Data at pH 7 (protein/α-ZrP) were shown for comparison.

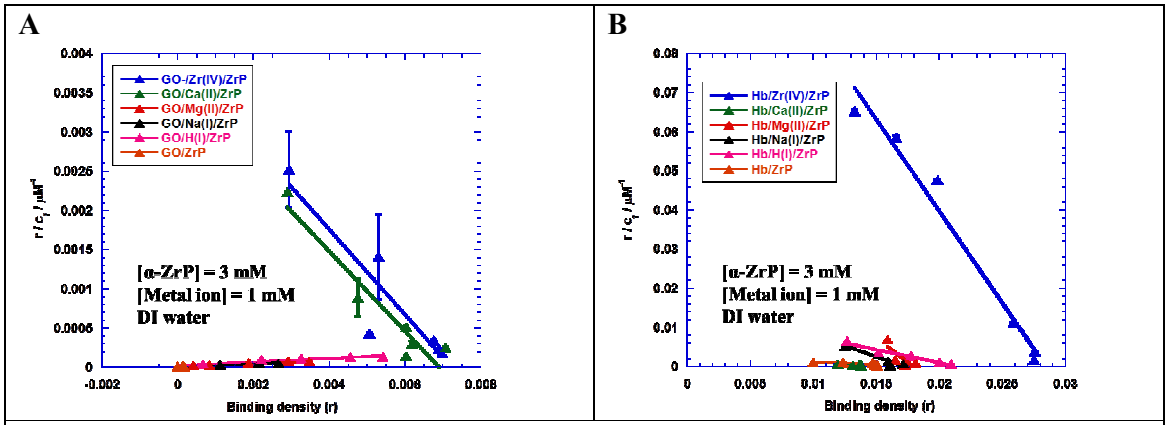

Figure S2: Schatchard Plots of specific enzyme/metal/α-ZrP systems. The pH was 3.0 in all cases except for the ones denoted as GO/α-ZrP and Hb/α-ZrP (pH ~6.0).

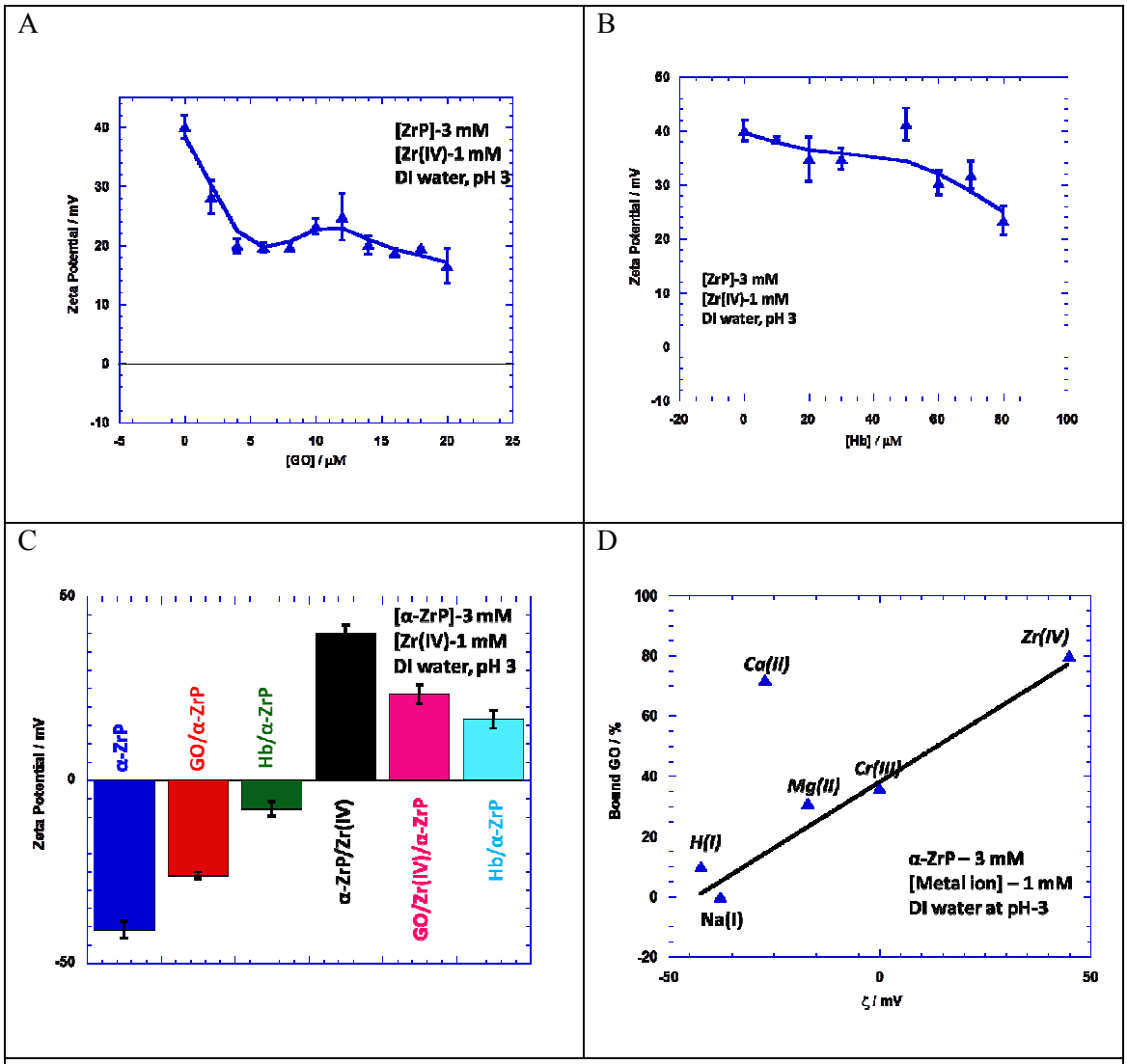

Figure S3: Variation of zeta potential by the addition of 2 µM increments of GO in (A) and 10 µM increments of Hb (B) to Zr(IV) activated α-ZrP; (C) Zeta Potential of intercalated systems at binding saturation. α-ZrP, Zr(IV) bound to α-ZrP, GO/Zr(IV)/ α-ZrP, GO/α-ZrP,, Hb/Zr(IV)/ α-ZrP and Hb/α-ZrP, 3 mM α-ZrP, 1 mM Zr(IV) , 20 µM GO and 80 µM Hb. All analysis has done in DI water at pH 3.0. (D) Correlation of protein binding with the net charge for GO/metal/α-ZrP. Only Ca(II) deviated from the plot, which suggested special role of Ca(II) in enhancing protein binding without charge reversal.

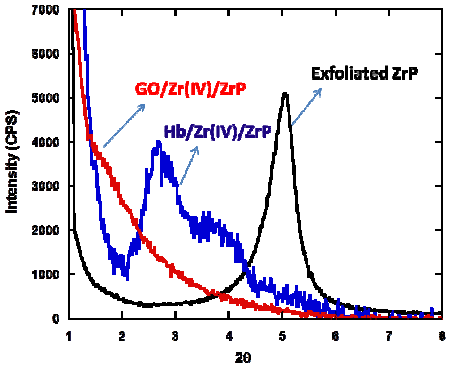

Figure S4: XRD of Metal/ enzyme–α-ZrP films showing the difference in spacing. Exfoliated α-ZrP showed a d value of 16 Å. Hb/Zr(IV)/α-ZrP showed 31 Å and Go/Zr(IV)/α-ZrP showed a broad peak around 51 Å. Hb/Zr(IV)/α-ZrP data is multiplied by factor of 10 and normalized. Hb has average radius around 60 Å. So we assume that the diffraction is second order.

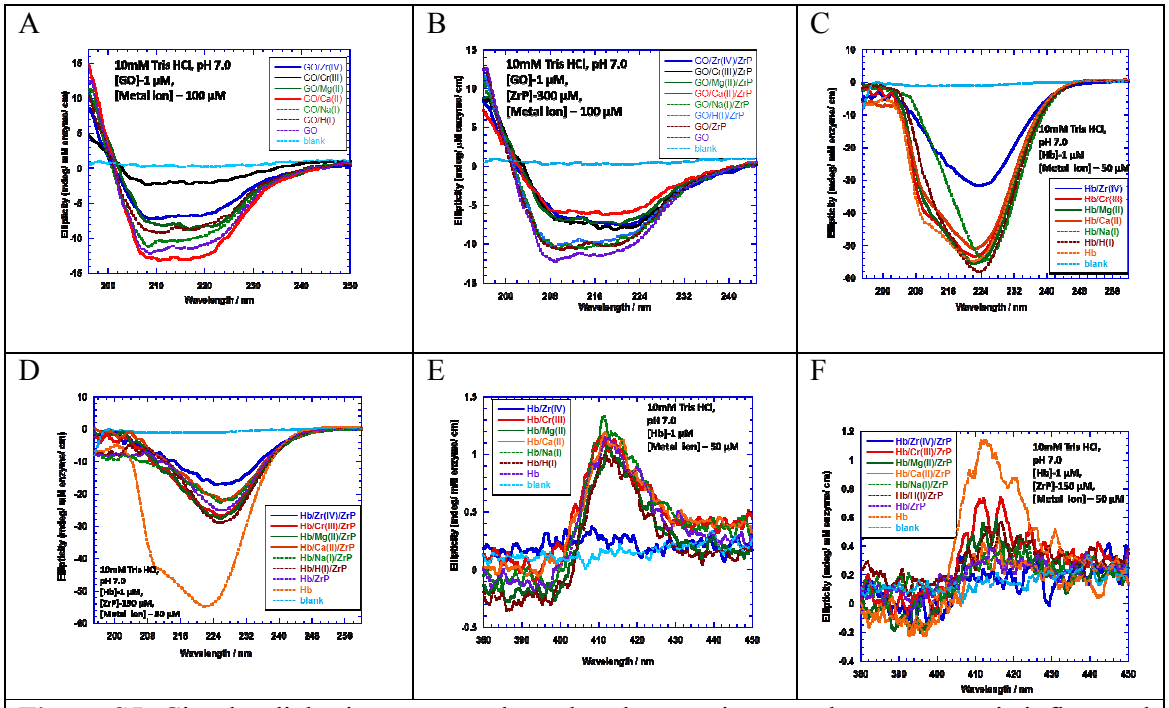

Figure S5: Circular dichroism spectra show that the protein secondary structure is influenced by the metal ions, before and after intercalation to a significant extent. **A and B:** CD spectra of GO/metal and GO/metal/ZrP; **C & D:** the CD spectra of Hb/metal and Hb/metal/ZrP which show significant distortion of Hb secondary structure; **E & F**: the soret CD spectra of Hb/metal and Hb/metal/ZrP which show that except Zr(IV), other metal ions did not distort the Soret band to a significant extent but on binding to the solid, the Soret CD is severely distorted, except in the case of Ca(II). All analysis were done in 10 mM Tris-HCl at pH 7.0 and 25^{o}C.

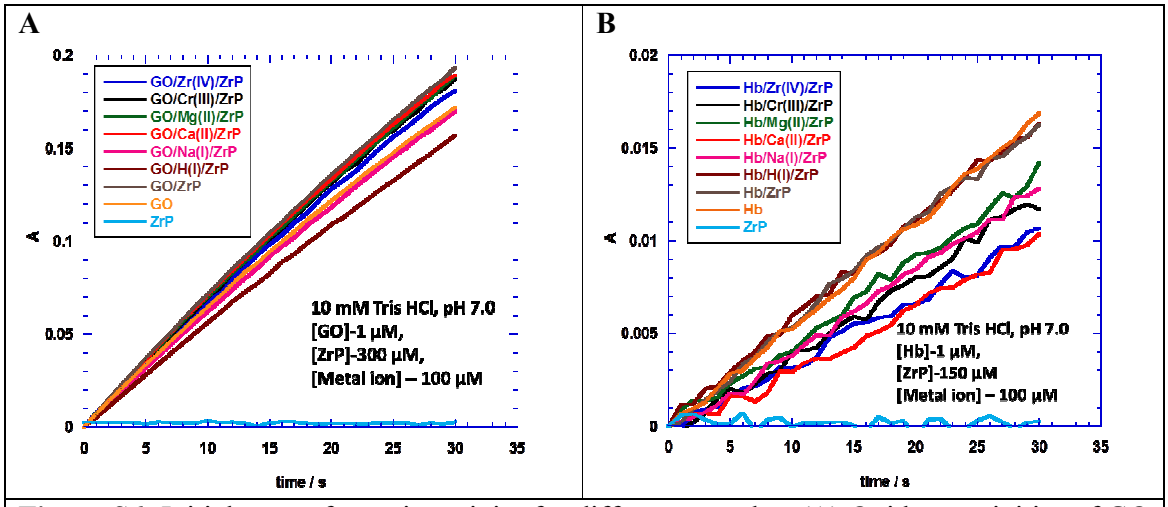

Figure S6: Initial rates of protein activity for different samples. **(A)** Oxidase activities of GO and GO/metal/α-ZrP and GO/α-ZrP. (B).Peroxidase activity of Hb, Hb/metal/α-ZrP and Hb/α-ZrP. All data are recorded in 10 mM Tris HCl buffer pH 7.0 buffer at 25°C after carrying out binding in DI water. At some conditions intercalation was so poor to measure the activity that data not shown. Oxidation state of the Metal ions is not shown in the legend because of the limited space in the graph.

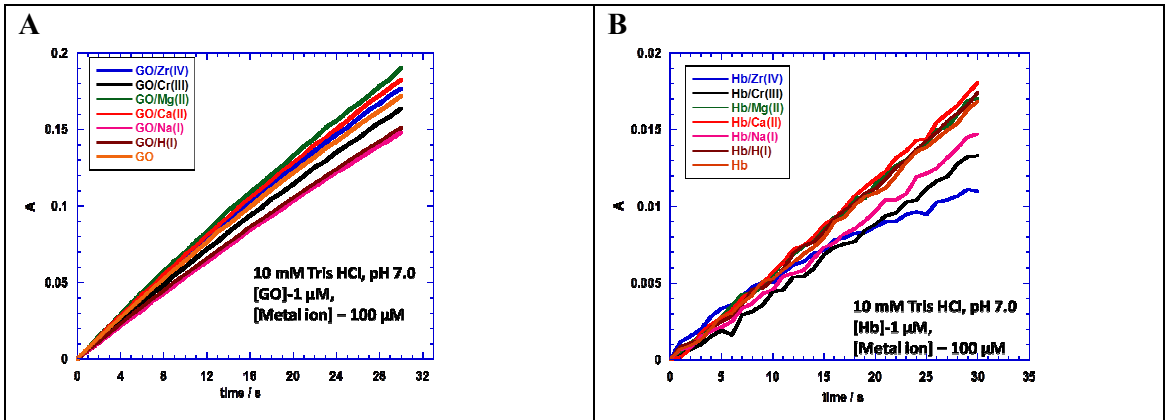

Figure S7: (A) Initial rates of GO (1 µM) activity with metal ions (100 µM) in 10 mM Tris HCl (**B**) Hb activity under same conditions as GO. It is clear that the metals did not inhibit enzyme activity. All data were recorded in 10 mM Tris HCl buffer pH 7.0 at 25°C. Protein/metal solutions were kept for an hour before measuring their activities. The activities of protein/metal/□-ZrP samples are determined by ressported assays, and initial activities calculated from the initial slope of the line during the first 30 seconds. Activities of physical mixtures of metal and the protein were also checked to evaluate the inhibition of activity by the metal, if any.

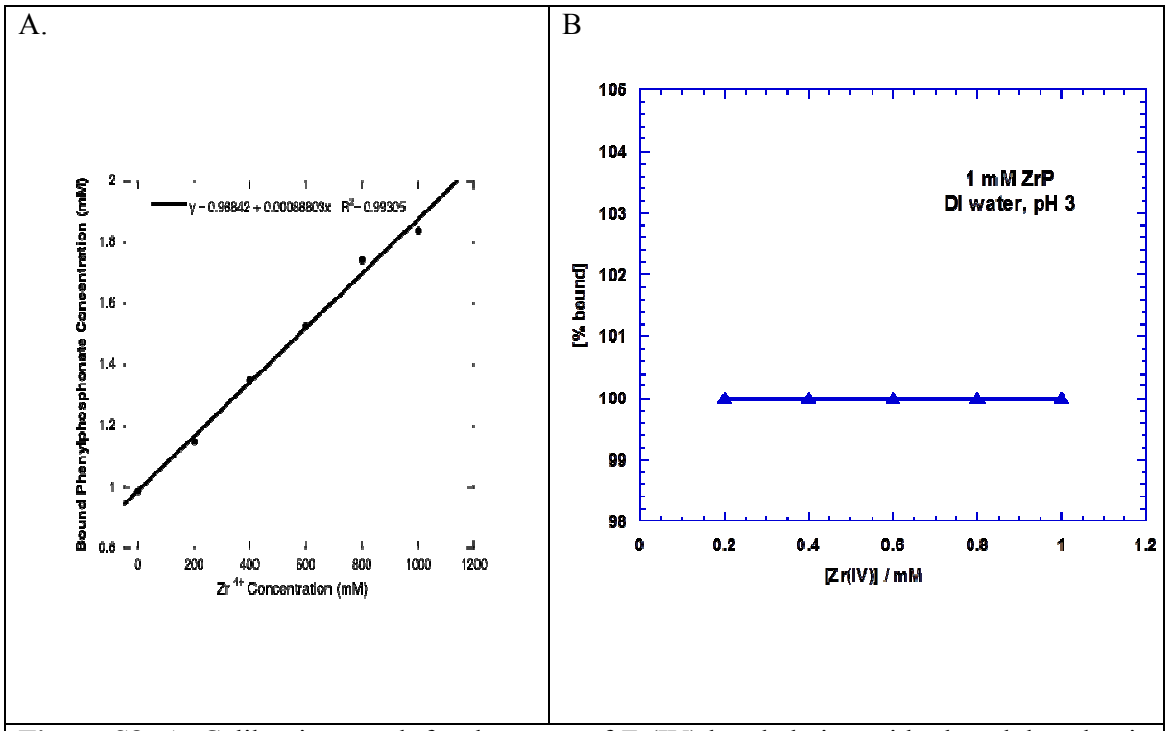

Figure S8: A. Calibration graph for the assay of Zr(IV) by chelation with phenylphosphonic acid. **B.** Binding of Zr(IV) to α-ZrP at pH 3 in DI water, shows 100% binding of the metal ion to the solid. **Assay for Zr(IV):** Phenyl phosphonic acid (2 mM) was used to determine the concentration of Zr(IV) bound in Zr(IV)/α-ZrP complexes. To construct a calibration curve, a series of samples were prepared by varying the concentration of Zr(IV) from 0-1 mM (0, 0.2, 0.4, 0.6, 0.8, 1.0) mM Phenyl phosphonic acid (2 mM) was added to each sample by keeping the total volume constant at 1ml. Samples were mixed, equilibrated for 3 h at room temperature and spun at 13000 rpm for 15 min to separate Z(IV) phenylphosphonate from free phenyl phosphonate. The absorbances of supernatants were checked for free phenylphosphonic acid at 263 nm with a measured extinction coefficient of 740 M^{-1} cm^{-1}. The calibration plot was constructed from this data as a function of known concentrations of Zr(IV). Based on our analysis of the supernatants, there was no free Zr(IV) in any of these samples.

Table S1: ***Binding constants and number of phosphate groups coordinated per protein***
Values obtained from **Figure S3.** Some data did not fit well and the corresponding binding constants were not estimated.

System	Binding constant K_b (10^5 /$M^{/1}$)	Number of phosphate groups, n	Maximum Loading (w/w %) Protein to α-ZrP
GO/Zr(IV)/ α-ZrP	5.4	140	100
GO/Ca(II)/Z α-ZrP	5.1	145	100
GO/ α-ZrP	0.014	412	40

Hb/Zr(IV)/ α-ZrP	46.9	35	400
Hb/Ca(II)/ α-ZrP	7.1	73	220
Hb/Mg(II)/ α-ZrP	24.5	56	250
Hb/Na(I)/ α-ZrP	11.2	60	240
Hb/ α-ZrP @pH 3	6.6	47	300
Hb/ α-ZrP	1.0	50	200

Table S2*: Binding assay, and* sample preparation in detail. Samples for binding studies prepared in a total volume of 1 mL. Metal ion solution is added to the protein solution and then exfoliated α-ZrP was added. Binding at pH 3 (H(I)) has been done using 100 mM HCl instead of metal. The mixture kept for 30 minutes for complete binding. Then centrifuged and separated bound enzyme from the free form. The volumes of reagents used for each set of data are shown in the corresponding table. All analyses have been done in triplicates and the average values are reported. The following Tables provide the exact compositions used.

A) GO/Zr(IV)/α-ZrP

[GO] (μM)	90 μM GO (μL)	100 mM $ZrOCl_2$ (μL)	60 mM ZrP (μL)	DI water (μL)	[Free GO] (μM)	[Bound GO] (μM)
10	110	10	50	830	1.19	8.80
20	220	10	50	720	4.10	15.8
30	330	10	50	610	11.8	18.2
40	440	10	50	500	19.7	20.2
50	550	10	50	390	29.3	20.7
60	660	10	50	280	39.0	20.9

B) GO/Ca(II)/α-ZrP

[GO] (μM)	90 μM GO (μL)	100 mM $CaCl_2$ (μL)	60 mM ZrP (μL)	DI water (μL)	[Free GO] (μM)	[Bound GO] (μM)
10	110	10	50	830	1.3	8.7
20	220	10	50	720	5.7	14.3
30	330	10	50	610	11.9	18.1
40	440	10	50	500	21.5	18.5
50	550	10	50	390	28.8	21.2
60	660	10	50	280	41.9	18.1

C) GO/Mg(II)/α-ZrP

[GO] (μM)	90 μM GO (μL)	100 mM $MgCl_2$ (μL)	60 mM ZrP (μL)	DI water (μL)	[Free GO] (μM)	[Bound GO] (μM)
10	110	10	50	830	7.73	2.26
20	220	10	50	720	13.7	6.23

30	330	10	50	610	20.4	9.56
40	440	10	50	500	27.1	12.8
50	550	10	50	390	33.6	16.3
60	660	10	50	280	41.6	18.3

D) GO/Na(I)/α-ZrP

[GO] (μM)	90 μM GO (μL)	100 mM NaCl (μL)	60 mM ZrP (μL)	DI water (μL)	[Free GO] (μM)	[Bound GO] (μM)
10	110	10	50	830	10.0	0.00
20	220	10	50	720	20.0	0.00
30	330	10	50	610	30.0	0.00
40	440	10	50	500	36.6	3.36
50	550	10	50	390	43.5	6.43
60	660	10	50	280	52.0	8.00

E) GO/H(I)/α-ZrP ; *HCl is added to adjust the pH to 3, so that the volume of water is also adjusted in each case.*

[GO] (μM)	90 μM GO (μL)	60 mM ZrP (μL)	[Free GO] (μM)	[Bound GO] (μM)
10	110	50	10.0	0.00
20	220	50	18.0	2.00
30	330	50	23.3	6.63
40	440	50	30.2	9.80
50	550	50	36.3	13.6
60	660	50	43.7	16.2

F) GO/α-ZrP

[GO] (μM)	90 μM GO (μL)	60 mM ZrP (μL)	DI water (μL)	[Free GO] (μM)	[Bound GO] (μM)
10	110	50	840	10.0	0.00
20	220	50	730	19.7	0.26
30	330	50	620	28.3	1.66
40	440	50	510	36.0	3.93
50	550	50	400	44.6	5.40
60	660	50	290	52.1	7.86

Similarly binding assay of Hb has also done. As shown below

G) Hb/Zr(IV)/α-ZrP

[Hb] (μM)	135 μM Hb (μL)	100 mM $ZrOCl_2$ (μL)	60 mM ZrP (μL)	DI water (μL)	[Free Hb] (μM)	[Bound Hb] (μM)
10	74	10	50	866	9.92	0.076
20	148	10	50	792	19.8	0.130
30	222	10	50	718	29.8	0.173
40	296	10	50	644	39.7	0.203
50	370	10	50	570	49.7	0.283
60	444	10	50	496	59.5	0.417
80	592	10	50	348	77.6	2.32
90	666	10	50	274	82.7	7.30
100	740	10	50	200	82.5	17.5

H) Hb/Ca(II)/α-ZrP

[Hb] (μM)	135 μM Hb (μL)	100 mM $CaCl_2$ (μL)	60 mM ZrP (μL)	DI water (μL)	[Free Hb] (μM)	[Bound Hb] (μM)
20	148	10	50	792	0.913	19.1
40	296	10	50	644	8.57	31.4
60	444	10	50	496	14.4	45.6
70	518	10	50	422	17.0	53.0
80	592	10	50	348	33.4	46.6
90	666	10	50	274	37.5	52.5
100	740	10	50	200	41.9	48.1

I) Hb/Mg(II)/α-ZrP

[Hb] (μM)	135 μM Hb (μL)	100 mM $MgCl_2$ (μL)	60 mM ZrP (μL)	DI water (μL)	[Free Hb] (μM)	[Bound Hb] (μM)
10	74	10	50	866	0.063	9.93
20	148	10	50	792	0.087	19.9
30	222	10	50	718	0.120	29.8
40	296	10	50	644	0.170	39.8
50	370	10	50	570	2.33	47.6
60	444	10	50	496	10.5	49.5
80	592	10	50	348	25.6	54.4
100	740	10	50	200	48.2	51.7

J) Hb/Na(I)/α-ZrP

[Hb] (μM)	135 μM Hb (μL)	100 mM NaCl (μL)	60 mM ZrP (μL)	DI water (μL)	[Free Hb] (μM)	[Bound Hb] (μM)
10	74	10	50	866	0.23	9.76
20	148	10	50	792	0.97	19.0

30	222	10	50	718	1.40	28.5
40	296	10	50	644	2.40	37.6
50	370	10	50	570	4.6	45.4
60	444	10	50	496	12.2	47.8
80	592	10	50	348	28.6	51.4
100	740	10	50	200	51.7	48.3

K) Hb/H(I)/α-ZrP

[Hb] (μM)	135 μM Hb (μL)	60 mM ZrP (μL)	[Free Hb] (μM)	[Bound Hb] (μM)
10	74	50	0.473	9.52
20	148	50	0.390	19.6
30	222	50	4.36	25.6
40	296	50	1.99	38.0
50	370	50	4.47	45.5
60	444	50	6.82	53.1
80	592	50	19.9	60.1
100	740	50	37.2	62.8

L) Hb/α-ZrP

[Hb] (μM)	135 μM Hb (μL)	60 mM ZrP (μL)	DI water (μL)	[Free Hb] (μM)	[Bound Hb] (μM)
10	74	50	876	2.62	7.37
20	148	50	802	5.35	14.6
30	222	50	728	6.74	23.2
40	296	50	654	10.0	29.9
50	370	50	580	12.9	37.1
60	444	50	506	15.9	44.1
80	592	50	358	35.3	44.6
100	740	50	210	54.8	45.1

Table S3: Key properties of metal ions[1].

Cation	Source	Ionic radii (pm)	Hydrated ion radii (pm)/charge
Zr(IV)	$ZrOCl_2$	80	112
Cr(III)	$CrCl_3$	76	150
Ca(II)	$CaCl_2$	70	150
Mg(II)	$MgCl_2$	45	200
Na(I)	NaCl	50	230
H^+	HCl	0.1	450

[1] Kielland, J., Individual activity coefficients of ions in aqueous solutions, *J. Am. Chem. Soc. 1937,* 59:1675-1678,