Supplementary Information

Plastic recognition and electrogenic uniport translocation of 1st-, 2nd- and 3rd-row transition and post-transition metals by transmembrane P_{1B-2}-type ATPase pumps

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Supplementary Figures

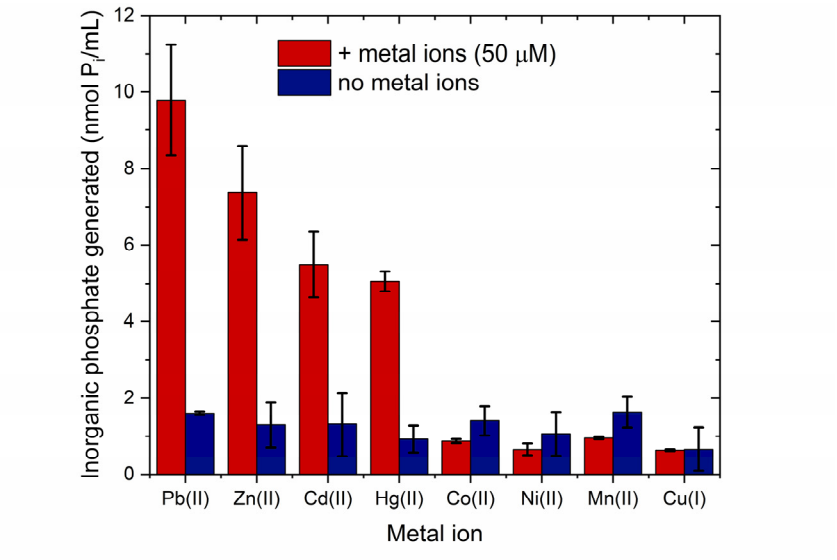

Figure S1: Background Pi and metal-independent ATPase activity.

Metal-stimulated *Cm*ΔZntA ATPase activity in the presence of different metals in Cymal-7 micelles (metal concentration = 50 μM; 30 min, 30 °C), compared to the corresponding background Pi generation in the absence of metals. Upon subtracting the Pi originating form buffer and protein independent ATP hydrolysis (approx. 10 nmol Pi/ml) only a minimal metal-independent background ATPase activity by *Cm*ΔZntA was observed (in average < 10% compared to the maximal activity, either due to uncoupling or background metal contamination). Thus, the metal-dependent stimulation of *Cm*ΔZntA ATPase activity can be reliably quantified (by subtracting the background in the absence of magnesium).

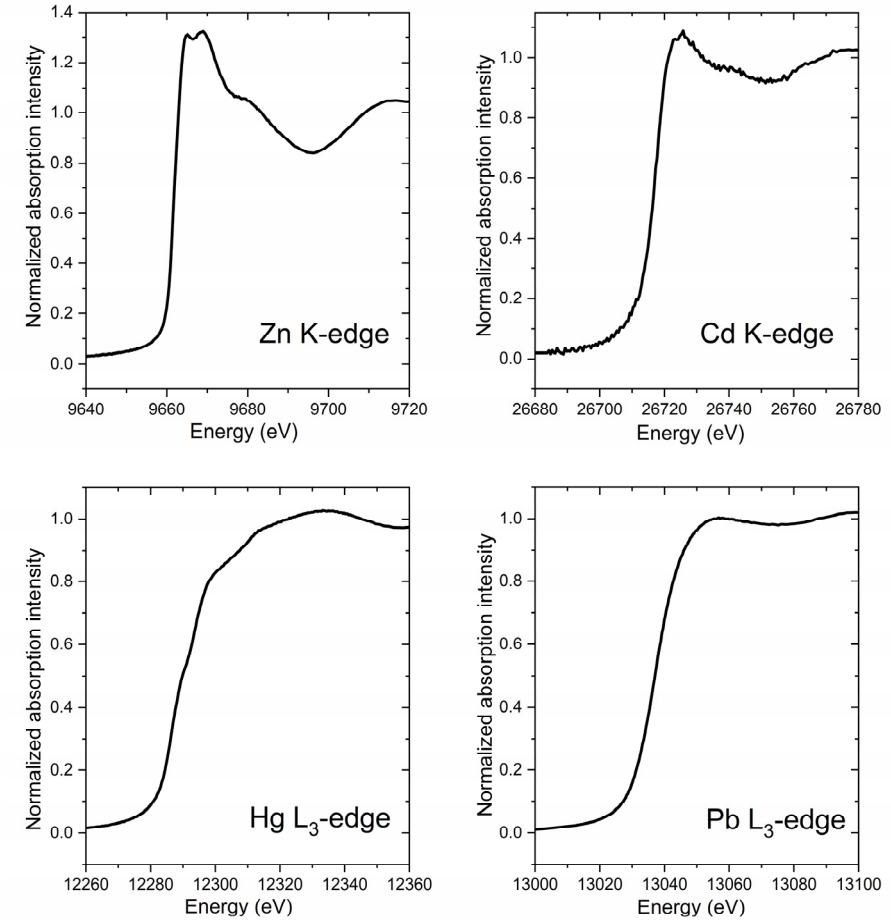

Figure S2: XANES spectra of *Cm*ΔZntA–M^{2+}.
XANES spectra of *Cm*ΔZntA–M^{2+} (M^{2+}= Zn^{2+}, Cd^{2+}, Hg^{2+} or Pb^{2+}) in Cymal-7 detergent micelles.

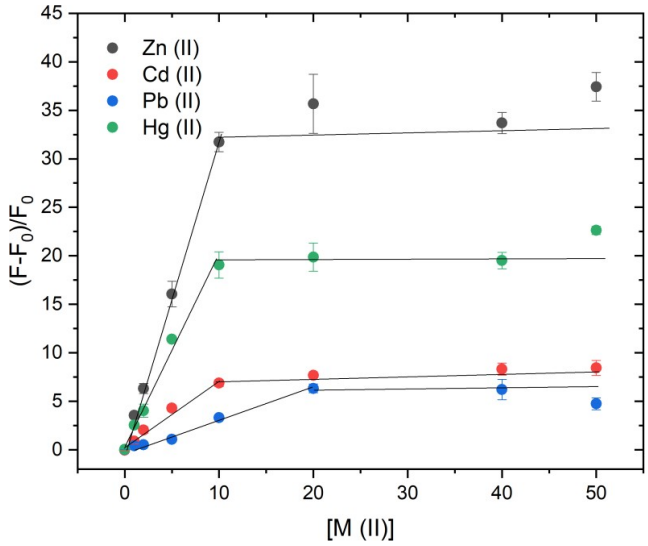

Figure S3: Titration plot of the metal fluorescent turn-on probes with the corresponding metal ions.

Fluozin-3 (10 µM) fluorescence turn-on response upon Zn^{2+} (black) or Cd^{2+} (red) titration (λ_{exc} = 480 nm; λ_{em} = 515 nm); Calcium green (10 µM) fluorescence response upon Hg^{2+} (green) titration (λ_{exc}= 506 nm ; λ_{em}= 531 nm), and Leadmium green (20 µM) fluorescence response upon Pb^{2+} (blue) titration (λ_{exc}= 490 nm; λ_{em}= 520 nm), reported as $(F–F_0)/F_0$ (with F_0 the fluorescence in the absence of metals), in the presence of 12.5 mg ml^{-1} liposomes (200nm). Data are mean ± s.d. (n=3).

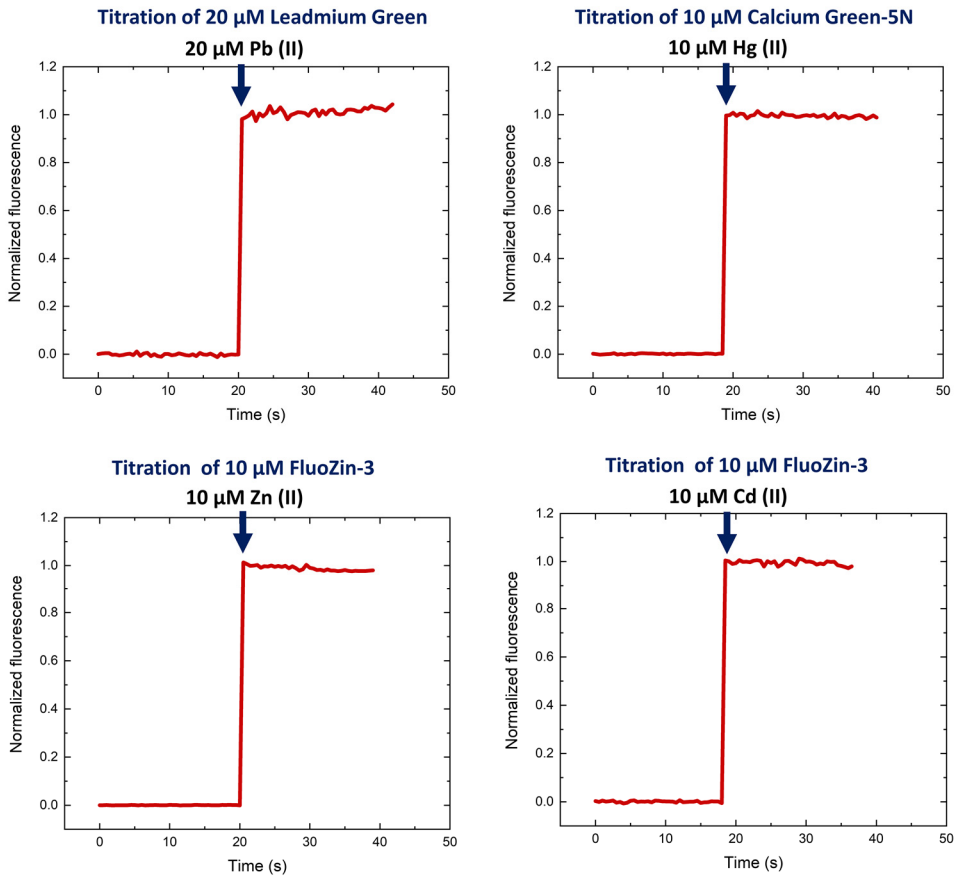

Figure S4: Kinetics of metal binding to the probes.
Fluorescence kinetic traces obtained upon titration of the fluorescent turn-on probes with Zn(II), Cd(II), Hg(II), and Pb(II). Complete stoichiometric binding occurs within the manual mixing-time upon metal addition (see arrow; approx. 5 seconds).

Table S1: Progression to best EXAFS fits for each metal-site

Shell	r (Å)	σ^2 (x10^{-3} Å^{-2})*	R-factor(%)	Red. χ^2
		Zn-site		
4N/O	2.11(2)	7(2)	27.5	184.7
4S	2.25(2)	10(1)	14.5	97.2
3N/O	2.03(1)	4(1)		
1S	2.31(1)	1(0)	4.12	35.1
2N/O	**1.99(0)**	**2(0)**		
2S	**2.29(0)**	**4(0)**	**2.91**	**24.8**
		Cd-site		
4N/O	2.46(0)	15(59)	98.80	327.41
2S	2.46(0)	1(0)	2.73	9.03
3S	2.46(0)	3(0)	1.48	4.93
4S	2.46(0)	5(0)	4.85	16.09
1N/O	2.24(2)	*negative*		
2S	2.53(2)	2(0)	1.21	5.01
2N/O	**2.32(2)**	**5(0)**		
2S	**2.48(2)**	**2(2)**	**1.73**	**6.4**
3N/O	2.30(1)	0(0)[a]		
1S	2.57(1)	2(2)	1.21	5.0
4N/O	**2.36(1)**	**1(0)**		
1S	**2.53(1)**	**3(1)**	**1.54**	**6.4**

3N/O	2.30(2)	25(11)		
2S	2.54(1)	1(1)	2.08	8.6
5N/O	2.37(0)	3(1)		
1S	2.51(1)	3(1)	2.39	9.8
4N/O	2.22(3)	29(11)		
2S	2.46(2)	1(0)	2.07	8.6
		Hg-site		
4N/O	2.43(0)	neg	4.64	18.7
5N/O	2.43(0)	0(0)	3.43	13.9
6N/O	2.43(0)	1(0)	3.93	15.9
2S	2.33(1)	3(0)	2.21	8.5
3S	2.33(1)	5(0)	8.7	33.3
1N/O	2.44(1)	0(2)[a]		
2S	2.33(1)	4(0)	1.53	7.5
1N/O	**2.48(3)**	**5(0)[b]**		
2S	**2.33(0)**	**3(0)**	**1.66**	**7.1**
2N/O	2.49(1)	0(2)[a]		
2S	2.31(1)	5(1)	3.10	15.1
2N/O	2.49(1)	5(0)[b]		
2S	2.31(1)	3(0)	3.31	15.1
		Pb-site		
2N/O	2.54(1)	1(1)	18.76	37.1

2S	2.67(2)	9(1)	8.06	15.9
3S	2.65(2)	9(0)	10.94	17.63
2N/O	2.48(6)	10(4)		
1S	2.67(1)	3(1)	8.50	20.5
1N/O	**2.52(2)**	**1(2)**		
2S	**2.67(2)**	**9(1)**	**7.34**	**17.7**
2N/O	2.54(2)	4(3)		
2S	2.65(2)	10(4)	7.92	19.1

r - inter atomic distance (in parentheses are mean-square deviations)
σ^2 - Debye-Waller factor (in parentheses are mean-square deviations in DW factor).
*** -** values reported as 0 represent small positive numbers after 3 significant figures (e.g. 0.003 with 0.002 error = 3(2); 0.0004 with 0.0001 error = 0(0))
[a] Unacceptable negative or null value
[b] σ^2 was fixed during fitting.

Table S2: Relative *Cm*ΔZntA ATPase activity and translocation "efficiency" observed for metal-stimulated ATPase hydrolysis ($V_{max}/K_{M,\ M2+}$) and metal translocation ($[\delta F/\delta t]/K_M$) in proteoliposomes, indicating the preferential order for metal substrates (Pb(II) > Zn(II) > Hg(II) ~ Cd(II)).

	$V_{max}/K_{M,\ M2+}$	**$[\delta F/\delta t]/K_M$**
Pb(II)	0.34	1.35
Zn(II)	0.15	0.29
Cd(II)	0.13	0.17
Hg(II)	0.12	0.14