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Supporting information of: A biomimetic cerium-based biosensor for the direct visual detection of phosphate under physiological conditions

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Ligand and complex synthesis:

The ligand synthesis of HXTA-H_5 and the formation of the complex $[Ce_2(HXTA)]^{3+}$ was performed using the described procedure by Que *et al*.[1]

[1]Branum, M. E., Tipton, A. K., Zhu, S., and Que, L. (2001). *J. Am. Chem. Soc,* **2001**, *123*, 1898–1904.

Assay:

The assay was performed with a TECAN safire 2 multiwell-plate spectrophotometer using magellan sofware to analyse spectra.

Stock solutions:

The following stock solutions should be prepared freshly (within minutes for CAN) before each round of experiments:

1) A stock solution of HEPES of 100 mM pH = 7. The pH of the solution is adjusted by solid NaOH.
2) A stock solution of 0.2 M of CAN, prepared by dissolving the appropriate amount of salt in HEPES 100 mM pH = 7
3) A stock solution of 0.1 M of HXTA-H_5, prepared by dissolving the appropriate amount of ligand in HEPES 100 mM pH = 7
4) A stock solution of 0.1 M of PCV, prepared by dissolving the appropriate amount of cathecol in HEPES 100 mM pH = 7
5) A stock solution of 0.1 M of Na_3PO_4, prepared by dissolving the appropriate amount of salt in HEPES 100 mM pH = 7

Working solutions:

Working solutions are prepared by a 100 x dilution in HEPES 100 mM pH = 7 (final concentration of compounds = 100 μM or 200 μM for the metal precusor). Working solutions are prepared each time for experiments. A 96 multiwell-plate is filled with working solutions.

Details of figure 2:

Well 1: 25 µL of PCV and 75 µL with HEPES buffer 100 mM pH=7

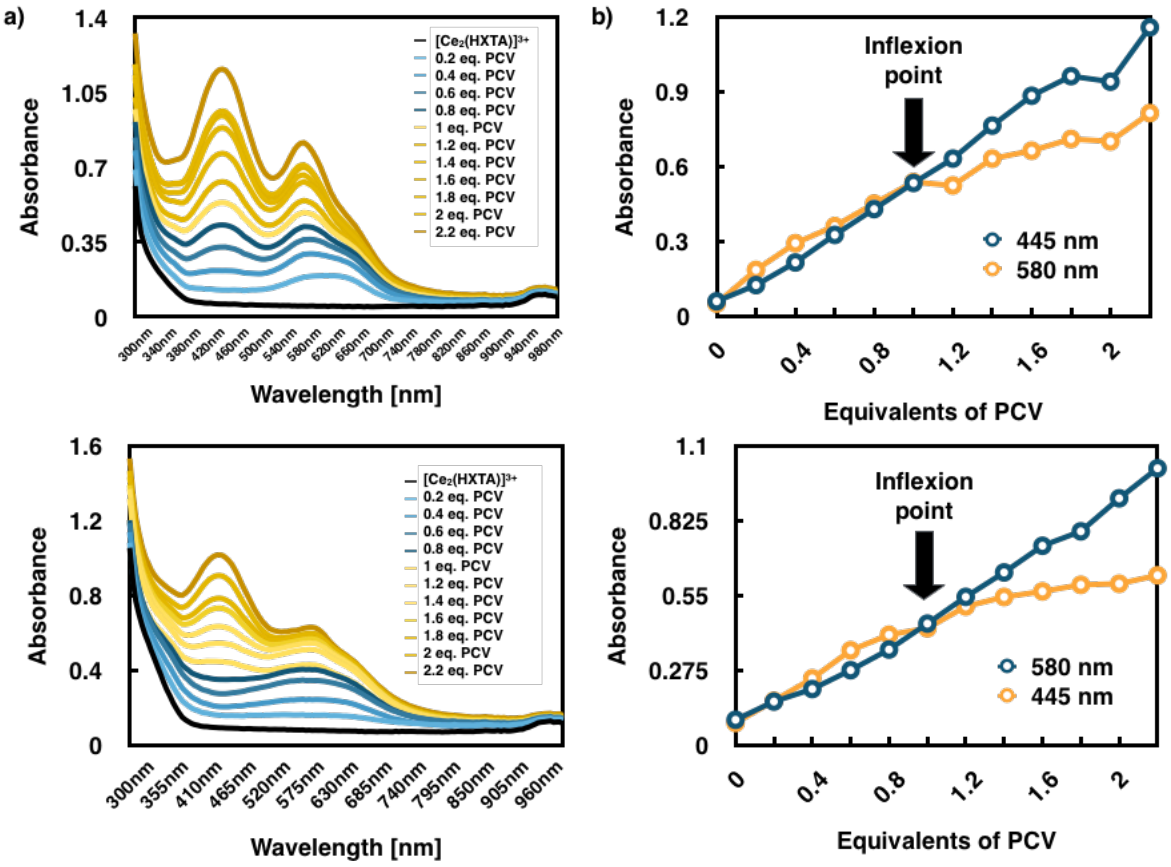
Well 2: 25 µL of CAN, 25 µL of HXTA-H_5 and 50 µL with HEPES buffer 100 mM pH=7

Well 3: 25 µL of CAN, 25 µL of HXTA-H_5, 25 µL of PCV, 25 µL and 25 µL with HEPES buffer 100 mM pH=7.4.

Well 4: 25 µL of CAN, 25 µL of HXTA-H_5, 25 µL of PCV, 25 µL of PO_4^{3-} .

A full scan is recorded for each well (with a spectrophotometer allowing comparison between the wells). Colorimetric differentiation can also be carried out with the naked-eye.

Duplicates of the figure 3:



Details of the figure 3:

A 96 well plate is filled as presented in the following table:

	CAN **Final:** 666 μM	**HXTA-H_5** **Final:** 333 μM	**PCV** **Final:** 0 - 916 μM	**HEPES** 100 mM pH = 7
Well 1	25 µL	25 µL	- (0 eq.)	25 µL
Well 2	25 µL	25 µL	0.5 µL (0.2 eq.)	24.5 µL
Well 3	25 µL	25 µL	1 µL (0.4 eq.)	24 µL
Well 4	25 µL	25 µL	1.5 µL (0.6 eq.)	23.5 µL
Well 5	25 µL	25 µL	2 µL (0.8 eq.)	23 µL
Well 6	25 µL	25 µL	2.5 µL (1. eq.)	22.5 µL
Well 7	25 µL	25 µL	3 µL (1.2 eq.)	22 µL
Well 8	25 µL	25 µL	3.5 µL (1.4 eq.)	21.5 µL
Well 9	25 µL	25 µL	4 µL (1.6 eq.)	21 µL
Well 10	25 µL	25 µL	4.5 µL (1.8 eq.)	20.5 µL
Well 11	25 µL	25 µL	5 µL (2 eq.)	20 µL
Well 12	25 µL	25 µL	5.5 µL (2.2 eq.)	19.5 µL

A full scan is recorded for each well.

Colorimetric differentiation can also be carried out with the naked-eye.

Details of the figure 4:

A 96 well plate is filled as presented in the following table:

	CAN **Final:** 500 μM	**HXTA-H_5** **Final:** 250 μM	**PCV** **Final:** 500-0 μM	**HEPES** 100 mM pH = 7
Well 1	25 µL	25 µL	50 µL	0 µL
Well 2	25 µL	25 µL	45 µL	5 µL
Well 3	25 µL	25 µL	40 µL	10 µL
Well 4	25 µL	25 µL	35 µL	15 µL
Well 5	25 µL	25 µL	30 µL	20 µL
Well 6	25 µL	25 µL	25 µL	25 µL
Well 7	25 µL	25 µL	20 µL	30 µL
Well 8	25 µL	25 µL	15 µL	35 µL
Well 9	25 µL	25 µL	10 µL	40 µL
Well 10	25 µL	25 µL	5 µL	45 µL
Well 11	25 µL	25 µL	0 µL	50 µL

A full scan is recorded for each well with a spectrophotometer (Tecan safire 2) allowing comparison between the wells. Colorimetric differentiation can also be carried out with the naked-eye.

Details of the figure 5:

Additional tests for the detection of anions at 10 μM of complex $[Ce_2(HXTA)]^{3+}$:

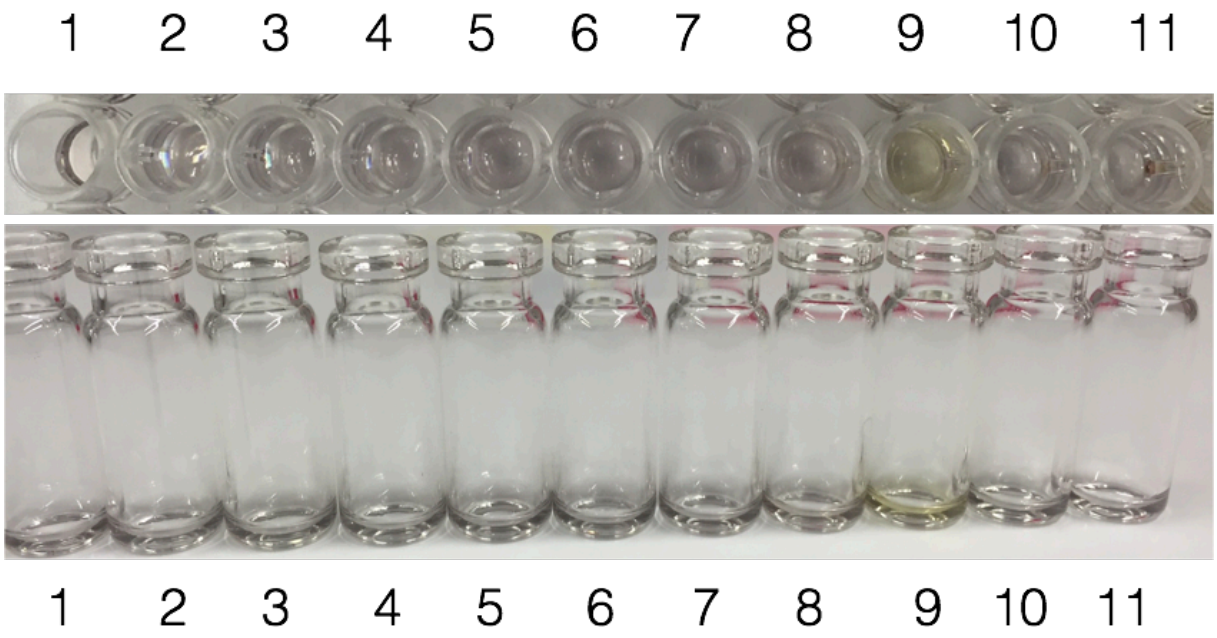
Preparation of $[(Ce_2(HXTA)(PCV)]^{+}$:

A stock solution of 0.01 M of $[(Ce_2(HXTA)(PCV)]^{+}$ is prepared by mixing: 500 µL of 0.01 M solution of ligand with 500 µL of 0.02 M solution of metal precursor and 500 µL of 0.01 M of PCV.

The stock solution is diluted 10 times with HEPES 100 mM pH=7 to form the working solution.

A 96 well plate is filled as presented in the following table:

	$[(Ce_2(HXTA)(PCV)]^{+}$ Final: 10 μM	**Anions** Final: 10 μM	**Anions**	**HEPES** 100 mM pH=7
Well 1	5 µL	15 µL	NaCl	30 µL
Well 2	5 µL	15 µL	NaBr	30 µL
Well 3	5 µL	15 µL	$NaNO_3$	30 µL
Well 4	5 µL	15 µL	$NaIO_3$	30 µL
Well 5	5 µL	15 µL	Na_2CO_3	30 µL
Well 6	5 µL	15 µL	HCOONa	30 µL
Well 7	5 µL	15 µL	CH_3COONa	30 µL
Well 8	5 µL	15 µL	Na Citrate	30 µL
Well 9	5 µL	15 µL	Na_3PO_4	30 µL
Well 10	5 µL	15 µL	Na salicylate	30 µL
Well 11	5 µL	15 µL	Na pyrophosphate	30 µL

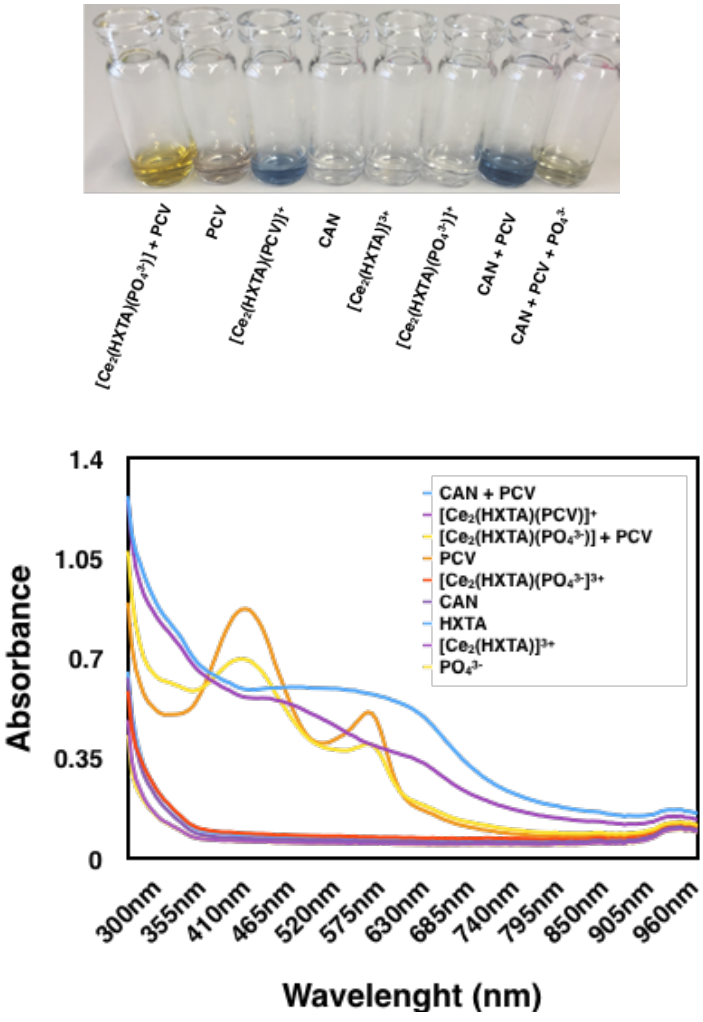


Picture 1: From left to right; NaCl (1), NaBr (2), $NaNO_3$ (3), $NaIO_3$ (4), Na_2CO_3 (5), HCOONa (6), CH_3COONa (7), Na citrate (8), Na_3PO_4 (9), Na salicylate (10), Na pyrophosphate (11).

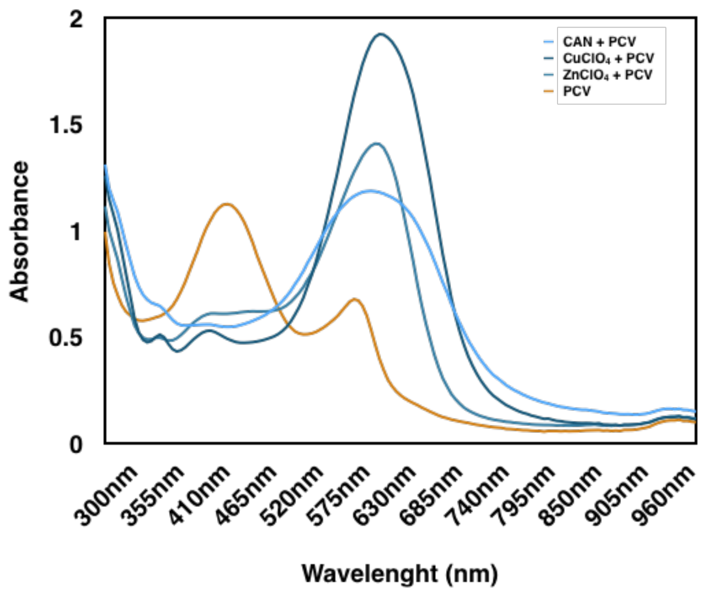
Details of the figure 6:

A 96 well plate is filled as presented in the following table:

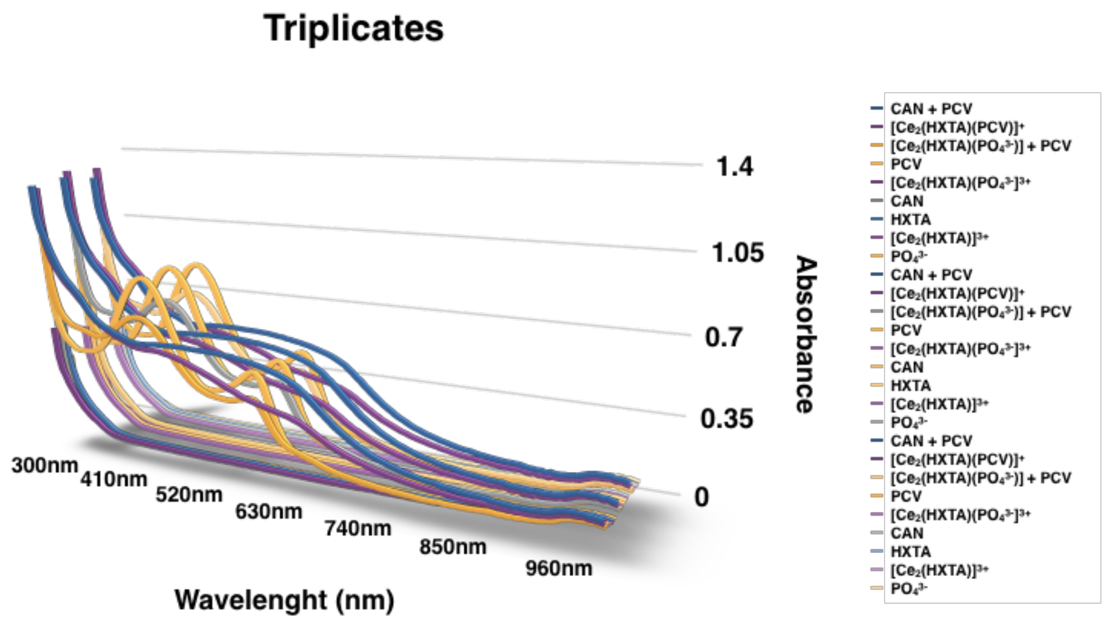
	[(Ce_2(HXTA)(PCV)]$^+$ Final: 250 µM	**PO_4^{3-} Final:** 500-0 µM	**HEPES** 100 mM pH = 7
Well 1	25 µL	50 µL	25 µL
Well 2	25 µL	45 µL	30 µL
Well 3	25 µL	40 µL	35 µL
Well 4	25 µL	35 µL	40 µL
Well 5	25 µL	30 µL	45 µL
Well 6	25 µL	25 µL	50 µL
Well 7	25 µL	20 µL	55 µL
Well 8	25 µL	15 µL	60 µL
Well 9	25 µL	10 µL	65 µL
Well 10	25 µL	5 µL	70 µL
Well 11	25 µL	0 µL	75 µL



Picture 1 : Additional controls



Picture 2 : Additional controls



Picture 3 : Triplicates of the figure 2

Affinity constant determination:

The determination of the k_a constant of $[(Ce_2(HXTA)(PCV)]^+$ was performed using described procedures in the supporting information by[2]

[2]Wenxiang Yu, Jian Qiang, Jun Yin, Srinivasulu Kambam, Fang Wang, Yong Wang and Xiaoqiang Chen *Org. Lett.*, **2014**, *16* (8), 2220–2223.

1) PCV titration

A stock solution of complex $[Ce_2(HXTA)]^{3+}$ is prepared as following:

200 µL of 0.001 M (100 x dilution) CAN in 0.1 M HEPES pH = 7 is mixed with
100 µL of HXTA of 0.001M (100 x dilution) in 0.1 M HEPES pH = 7

PCV solution:

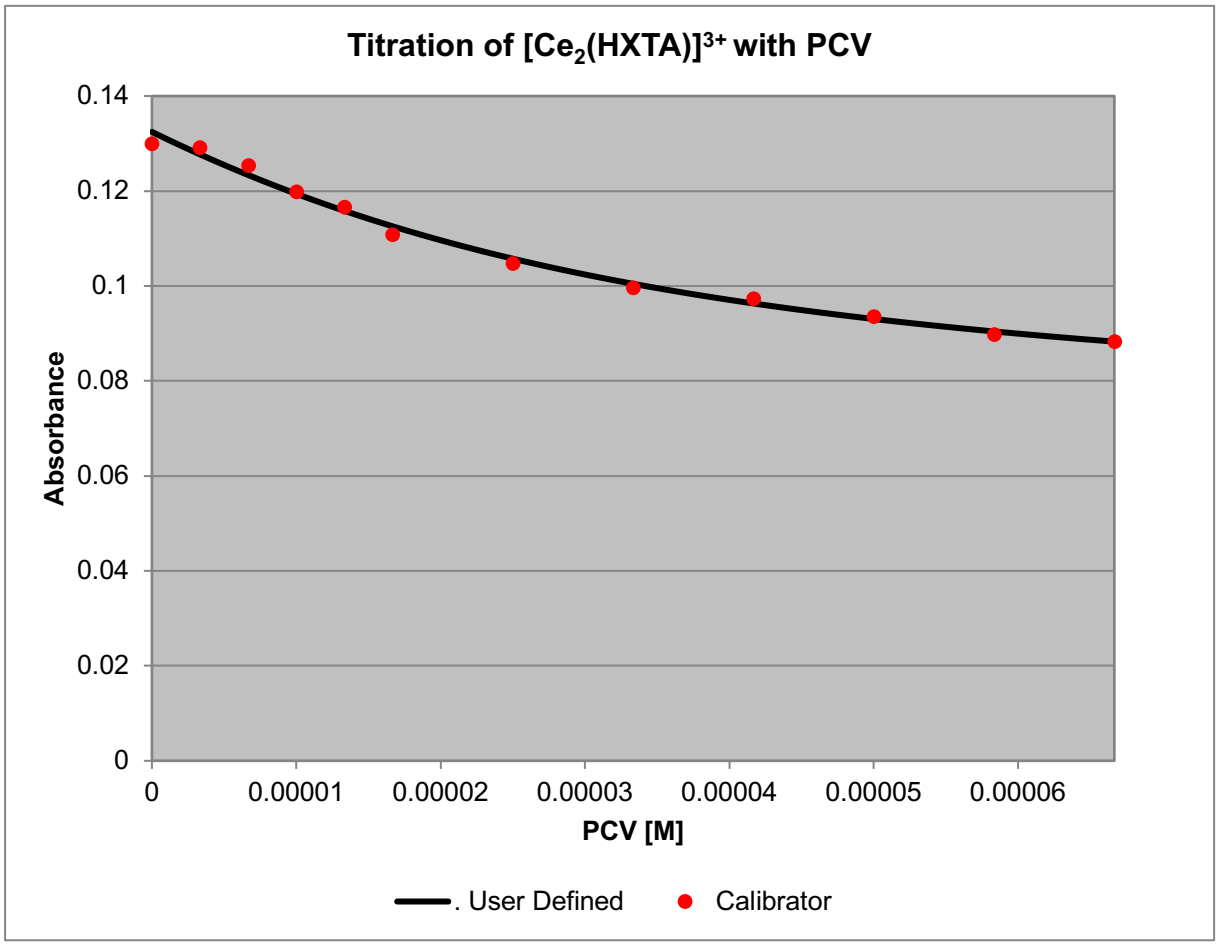
1000 µL of 0.0001 M of PCV (1000x dilution) in HEPES 0.1 M pH = 7

A 96 well plate is filled as following (3 times):

N°	Volume PCV [µL]	Volume $[Ce_2(HXTA)]^{3+}$ [µL]	Buffer volume [µL]	Total volume [µL]
Well 1	20	20	60	100
Well 2	20	17.5	62.5	100
Well 3	20	15	65	100
Well 4	20	12.5	67.5	100
Well 5	20	10	70	100
Well 6	20	7.5	72.5	100
Well 7	20	5	75	100
Well 8	20	4	76	100
Well 9	20	3	77	100
Well 10	20	2	78	100
Well 11	20	1	79	100
Well 12	20	0	80	100

Measurements:

N°	PCV (µM)	C (µM)	Measurements at 445 nm	**Measurements at 580 nm**
Well 1	20	66.67	0.0907	**0.13**
Well 2	20	58.33	0.1672	**0.1292**
Well 3	20	50.00	0.1057	**0.1254**
Well 4	20	41.67	0.0958	**0.1199**
Well 5	20	33.33	0.0867	**0.1166**
Well 6	20	25.00	0.0893	**0.1108**
Well 7	20	16.67	0.0958	**0.1048**
Well 8	20	13.33	0.1017	**0.0997**
Well 9	20	10.00	0.1097	**0.0974**
Well 10	20	6.67	0.1194	**0.0936**
Well 11	20	3.33	0.1317	**0.0898**
Well 12	20	0.00	0.1256	**0.0884**



Equation: Absorbance = n+a*sqrt((0.00002+x +1/K_a)^2-0.00008*x)+a*(0.00002-x-1/K_a)

Coeff.	Value	± Error
a	1575.54512518984	134.298582564086
k	44479.7316983058	12559.1660127683
n	0.0694256272576168	0.00582209059595647

Measure	Value
R^2	0.996298173335589

Calculated affinity constant using Stata SE: **K_a = 44479 M^{-1}**

2) Phosphate titration

A stock solution is prepared as following:

200 µL of 0.001 M (100x) CAN in 0.1 M HEPES pH = 7 is mixed with
100 µL of HXTA of 0.001M (100x) in 0.1 M HEPES pH = 7 is mixed with
100 µL of PCV of 0.001M (100x) in 0.1 M HEPES pH = 7 is mixed with

The stock solution is diluted 2.5 times.

Phosphate solution:

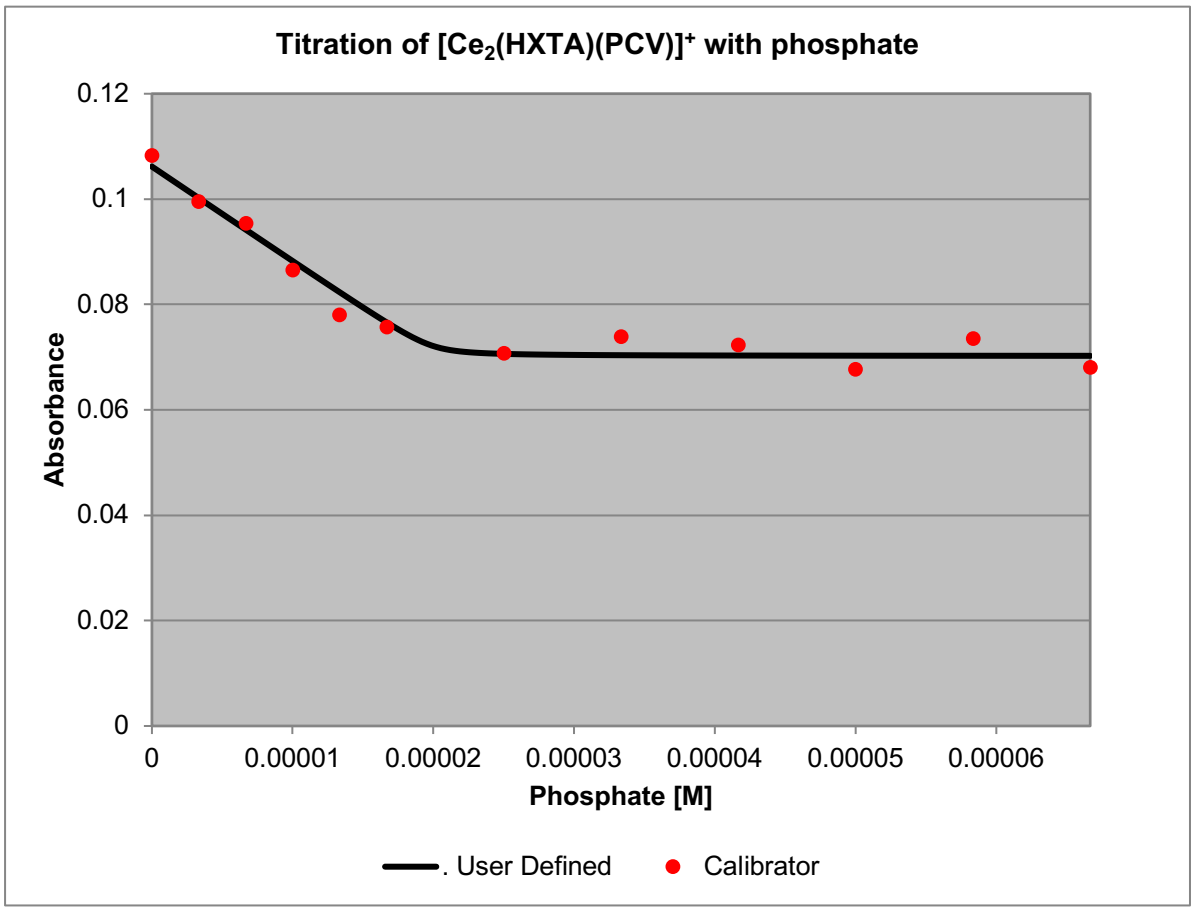
A 100x time dilution of phosphate in HEPES 0.1 M pH = 7

A 96 well plate is filled as following (3 times):

N°	Volume $[(Ce_2(HXTA)(PCV)]^+$ [µL]	Volume analyte [µL]	Buffer volume [µL]	Total volume [µL]
Well 1	20	20	60	100
Well 2	20	17.5	62.5	100
Well 3	20	15	65	100
Well 4	20	12.5	67.5	100
Well 5	20	10	70	100
Well 6	20	7.5	72.5	100
Well 7	20	5	75	100
Well 8	20	4	76	100
Well 9	20	3	77	100
Well 10	20	2	78	100
Well 11	20	1	79	100
Well 12	20	0	80	100

Measurements:

N°	PCV (µM)	$[(Ce_2(HXTA)(PCV)]^+$ (µM)	**Measurements at 445 [nm]**	Measurements at 580 [nm]
Well 1	20	66.67	**0.1083**	0.0684
Well 2	20	58.33	**0.0996**	0.0695
Well 3	20	50.00	**0.0954**	0.0694
Well 4	20	41.67	**0.0866**	0.0728
Well 5	20	33.33	**0.078**	0.0753
Well 6	20	25.00	**0.0757**	0.0715
Well 7	20	16.67	**0.0707**	0.079
Well 8	20	13.33	**0.0739**	0.0767
Well 9	20	10.00	**0.0723**	0.0914
Well 10	20	6.67	**0.0677**	0.0966
Well 11	20	3.33	**0.0736**	0.0977
Well 12	20	0.00	**0.0681**	0.1049



Equation: Absorbance = n+m*(0.00002+x-sqrt((0.00002-x)^2+0.00008*K_b*x))/(1-K_b)

Coeff.	Value	± Error
m	-899.062057237362	53.220816910875
k	0.0031894	0.0171249
n	0.106172817884357	0.0016777559231092

Measure	Value
R^2	0.966145577869551

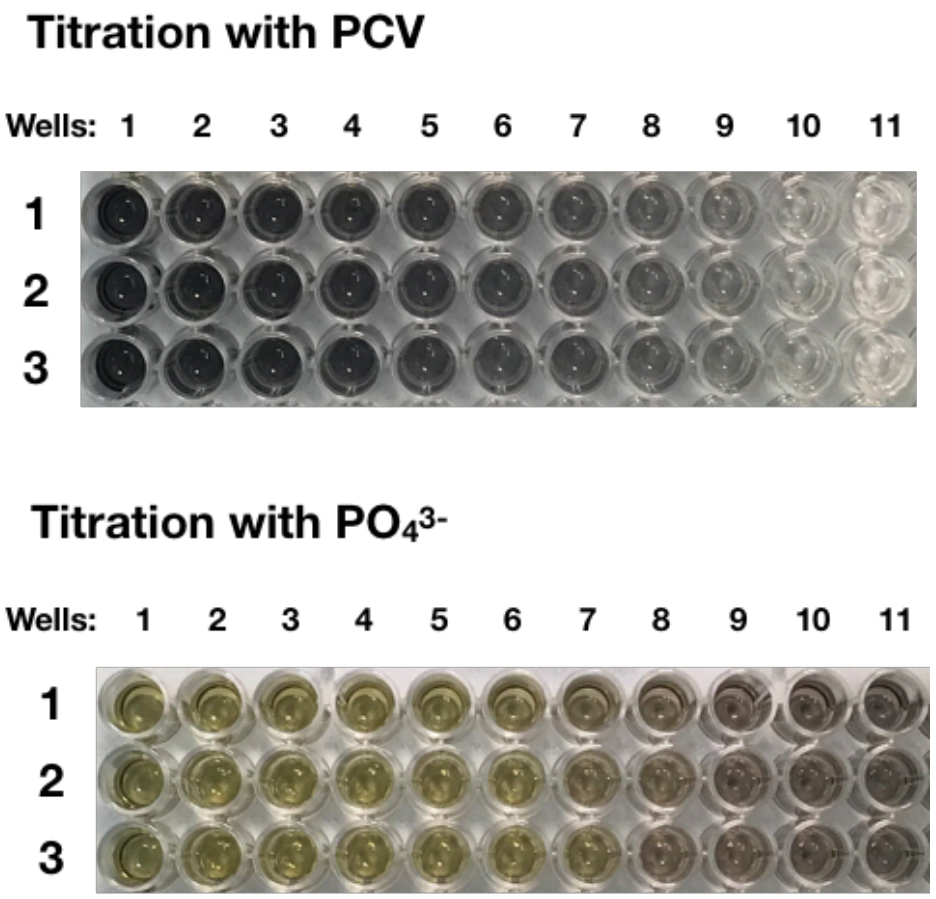
Calculated affinity constant using Stata SE: **K_b = 0.0031 M^{-1}**

Determination of the affinity constants:

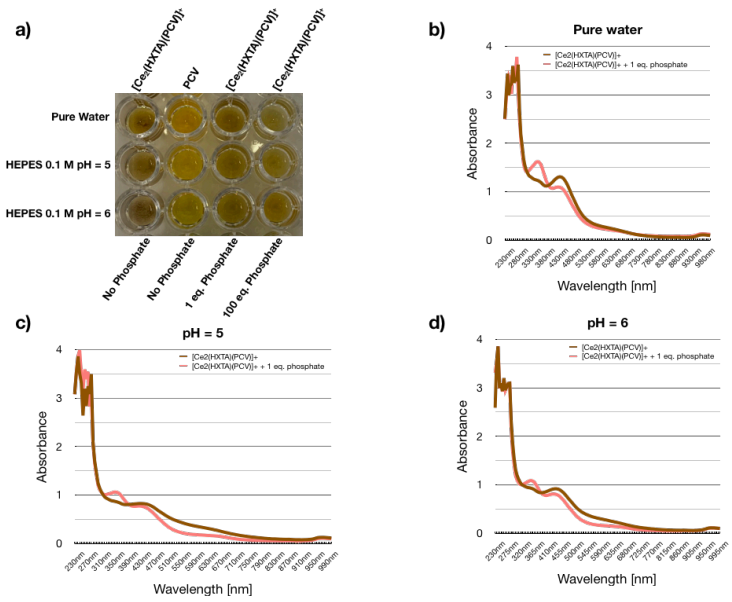
$$k = \frac{K_a}{K_b}$$

K_a = **4.479 x 10^4 M^{-1}**

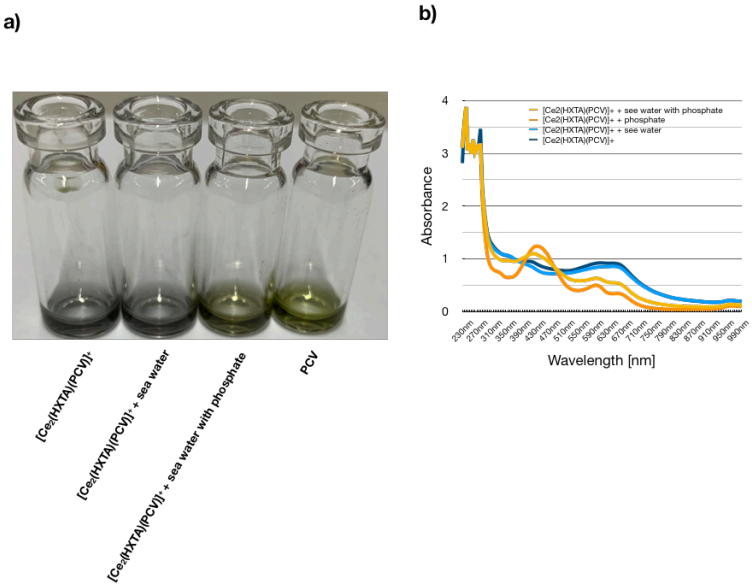
K_b = K_a / k = 44790 x 10^4/ 0.0031 = **1.44 x 10^7 M^{-1}**



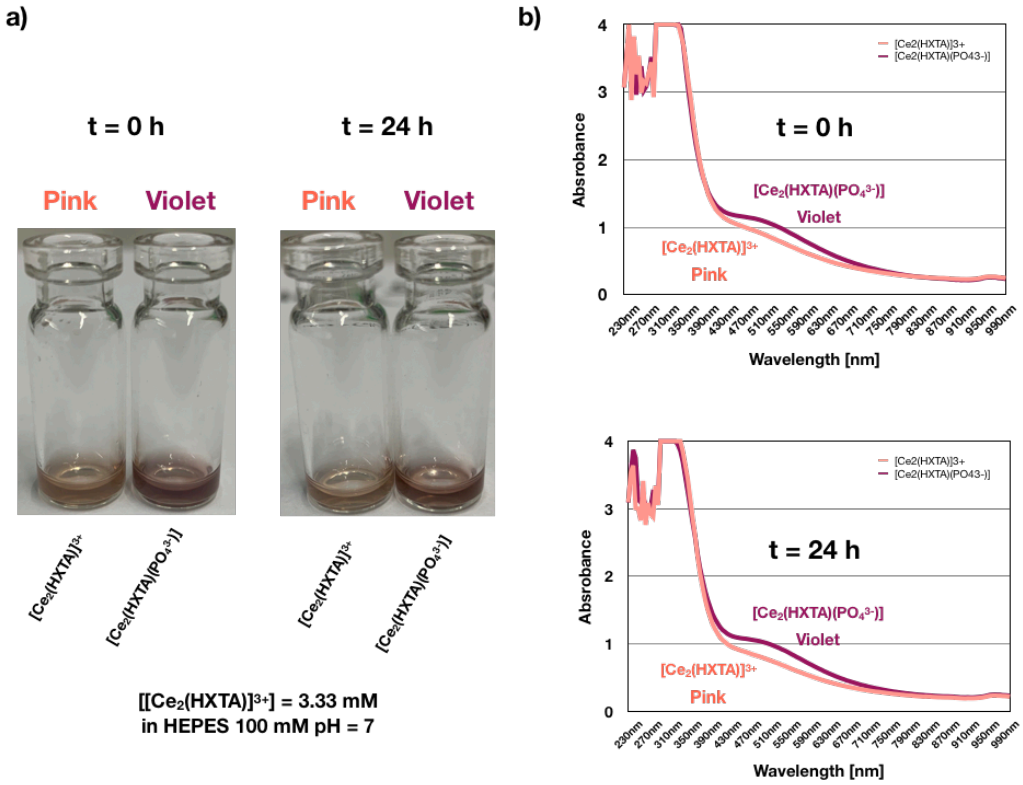
Picture 4: Visual results of the titration in triplicates



Picture 5: a) Screening for the detection phosphate with $[Ce_2(HXTA)(PCV)]^+$ (250 µM) at various pHs and in pure water. b) Absorbance spectra (in brown) of $[Ce_2(HXTA)(PCV)]^+$ and in the presence of 1 eq. of phosphate (in red) in pure Water c) Absorbance spectra (in brown) of $[Ce_2(HXTA)(PCV)]^+$ and in the presence of 1 eq. of phosphate (in red) at pH = 5 in HEPES 100 mM d) Absorbance spectra (in brown) of $[Ce_2(HXTA)(PCV)]^+$ and in the presence of 1 eq. of phosphate (in red) at pH = 6 in HEPES 100 mM.



Picture 6: a) addition of see water to the heteroleptic $[Ce_2(HXTA)(PCV)]^+$ (250 µM) doesn't perturb the sensor. Addition of see water spiked with phosphate (1 eq.) allows the recognition of phosphate in a complex medium b) Absorbance spectras of the addition of phosphate to the sensor; in dark blue the sensor alone (250 µM in HEPES 100 mM pH=7), in light blue the sensor in see water, in yellow the sensor in the presence of 1 eq. of phosphate and in orange the sensor in the presence of see water spiked with 1 eq. of phosphate.



Picture 7: a) addition of phosphate (1 eq.) to the homoleptic complex $[Ce_2(HXTA)]^{3+}$ (250 µM) increases the absorbance of the complex between 390 to 670 nm turning the color of the complex from pink to violet and can be seen with the naked-eye. The formed complexes are at least stable 24h. b) Absorbance spectras of $[Ce_2(HXTA)]^{3+}$ in absence of phosphate (pink) or in the presence of phosphate (violet). The complexes are stable at least 24h.

Table of the extinction coefficients at 2 wavelengths (425 nm and 595 nm):

	$A_{(595\ nm)}$	$A_{(425\ nm)}$	$\varepsilon_{(595\ nm)}$	$\varepsilon_{(425\ nm)}$
$[Ce_2(HXTA)]^{3+}$	0.1841	0.3225	736	1290
$[Ce_2(HXTA)]^{3+}$ + PCV + Phosphate	0.5370	1.1406	2148	4562
PCV	1.1469	2.8219	4587	11287
$[Ce_2(HXTA)]^{3+}$ + PCV	0.6750	0.7690	2700	3076