## **CHEMBIOCHEM**

## **Supporting Information**

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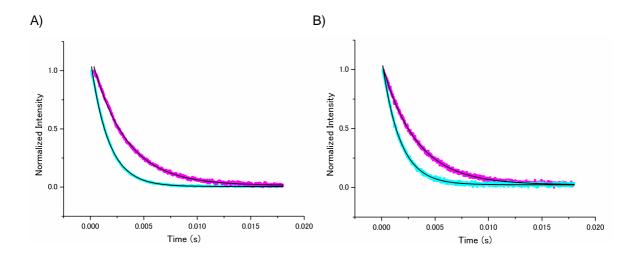
## **Supporting Information**

for

Selective Detection of Phosphotyrosine among Various Phosphate-Containing Biomolecules by Terbium(III) Complex

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Luminescence lifetime measurements and estimated q value: Luminescence lifetime measurements were conducted with Tb(III)-DOTAM containing H<sub>2</sub>O or D<sub>2</sub>O solutions (10 mm HEPES buffer, pH = 7 for H<sub>2</sub>O solution). Two ml of solution was excited at 355 nm by pulse laser from Nd:YAG lazer (Spectra-Physics KK. INDI-40-10-HG-TRI-T). Luminescence at 545 nm was measured by using a monochromator (JASCO CT-25TP) and a photomultiplier (Hamamatsu Photonics R446). In Figure S1 the measured decay curves and their fitted curves are shown.



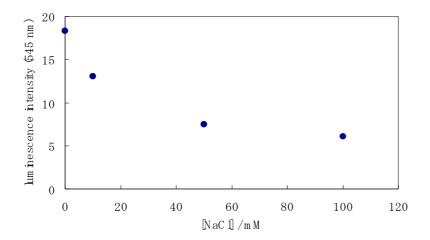
**Figure S1**. Luminescence decay profiles of A) Tb(III)-DOTAM complex and B) Tb(III)-DOTAM complex + phenyl phosphate (10 equivalence). Blue: measurements in  $H_2O$  solution; purple: measurements in  $D_2O$  solution. The fitting curve (black) was fitted as an exponential decay curve.

The estimation of q value of lanthanide complex in aqueous solution is reviewed in Ref. [14]. The q value (the number of coordinated water molecules) was calculated with following Equation (1):

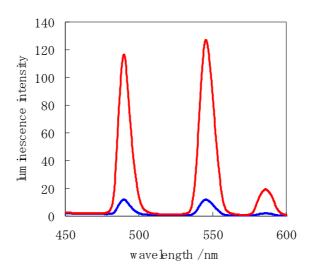
$$q = 5.0(k_{H,O} - k_{D,O} - 0.06)$$
 (1)

where  $k_{H_2O}$  and  $k_{D_2O}$  represents measured rate constant for solutions of H<sub>2</sub>O and D<sub>2</sub>O, respectively. The calculated half-life based on fitting curve and estimated q value is summarized in Table S1. The q value is uncertain in the range of ±0.5.

<b>Table S1</b> . The half-life $\tau$ and calculated $q$ value of Tb(III)-DOTAM complex.			
	$ au_{{\scriptscriptstyle H}_2O}$	$ au_{\scriptscriptstyle D_2O}$	
	(ms)	(ms)	q
_[a]	1.65	3.17	1.15
+phenyl phosphate	1.65	3.20	1.17
[a] The data in the absence of phenyl phosphate.			



**Figure S2**. Change in the luminescence by the addition of NaCl to the solution of Tb<sup>III</sup>-DOTAM and phenyl phosphate. Conditions: [Tb<sup>III</sup>-DOTAM] = [phenyl phosphate] = 100 μM in 10 mm HEPES buffer (pH = 7.0),  $\lambda_{ex}$  = 262.5 nm,  $\lambda_{em}$  = 545 nm.



**Figure S3**. Luminescence spectra of the solution of Tb<sup>III</sup>-DOTAM and phosphorylated (red line) or nonphosphorylated (blue line) v-Src substrate nonapeptide. Conditions: [Tb<sup>III</sup>-DOTAM] = [nonapeptide] = 100  $\mu$ M in 10 mM HEPES buffer (pH = 7.0),  $\lambda_{ex}$  = 262.5 nm.