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A selective Seoul-Fluor-based bioprobe, S*f*BP, for vaccinia H1-related phosphatase—a dual-specific protein tyrosine phosphatase†

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We report a Seoul-Fluor-based bioprobe, S*f*BP, for selective monitoring of protein tyrosine phosphatases (PTPs). A rational design based on the structures at the active site of dual-specific PTPs can enable S*f*BP to selectively monitor the activity of these PTPs with a 93-fold change in brightness. Moreover, screening results of S*f*BP against 30 classical PTPs and 35 dual-specific PTPs show that it is selective toward vaccinia H1-related (VHR) phosphatase, a dual-specific PTP (DUSP-3).

Protein phosphorylation is one of the major post-translational modification mechanisms that nature utilizes to control various signal transduction pathways. Specifically, many essential regulatory processes in signal cascades are controlled by tyrosine phosphorylation, whose homeostasis is modulated by the interplay between protein tyrosine phosphatases (PTPs) and protein tyrosine kinases (PTKs).[1] As observed in PTKs, the malfunction of PTPs has a serious correlation with many human diseases including cancers, diabetes, rheumatoid arthritis, and hypertension.[2] For instance, PTP1B is a major negative regulator of insulin signaling in muscle and liver, and the loss of PTP1B activity leads to enhanced insulin sensitivity and resistance to weight gain in mice, which implies that the development of selective inhibitors of PTP1B can provide a potential treatment for type 2 diabetes and/or obesity.[3] Therefore, the discovery of novel and specific small-molecule regulators of certain PTPs with distinct modes of action can provide an essential tool for the understanding of the molecular basis of PTP catalysis and substrate specificity. Despite their importance, the high degree of structural similarity between PTPs, which constitute a large family of enzymes, has hampered the development of selective inhibitors for specific PTPs.[4] In this regard, selective PTP probes can be useful tools to monitor the activity of specific PTPs, which define the structural difference and similarity at the active sites of various PTPs.[5] Class I human PTPs consist of classical PTPs and dual-specific PTPs (DUSPs). While classical PTPs have narrow and deep active sites (depth ~10 Å) to accommodate phosphotyrosine, DUSPs have wide and shallow active sites (depth = 4.5–6 Å).[6] On the basis of this structural information, we aimed to design and synthesize selective fluorescent probes for a single DUSP or a group of DUSPs employing *ortho*-substituted phenyl phosphate.[7] Herein, we report a highly selective fluorescent probe called S*f*BP (*S*eoul-*F*luor-based *B*ioprobe for specific *P*TP) for vaccinia H1-related (VHR) phosphatase.

Previously, we reported the discovery of a tunable and predictable fluorescent core skeleton, namely Seoul-Fluor,[8] and its subsequent application as a fluorescent bioprobe.[9] Because the photophysical properties of Seoul-Fluor have a significant correlation with the electron density of its substituents, we can rationally design sensitive bioprobes for specific biological events using this molecular frame. In other words,

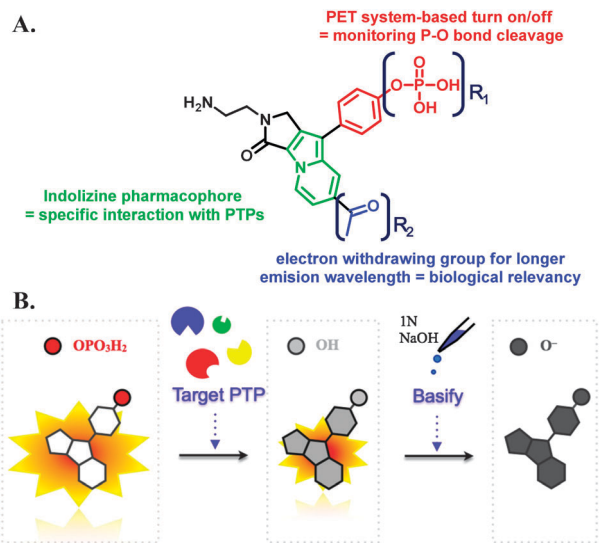

Fig. 1 (A) Designing principles of Seoul-Fluor-based bioprobes for specific PTPs (S*f*BP). (B) Schematic representation of the assay system used to monitor PTPs activity *via* the PeT-based sensing mechanism.

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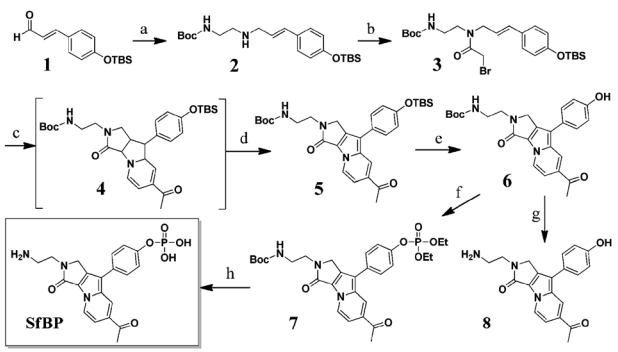
we can monitor the enzyme-mediated changes in electronic characteristics of substituents, which might alter the photo-induced electron transfer (PeT) process in Seoul-Fluor.[10] We also surmised that the Seoul-Fluor-based bioprobe can specifically interact with certain enzymes due to the presence of a well-known pharmacophore, indolizine, in the Seoul-Fluor core skeleton.[11]

On the basis of this hypothesis, we designed a Seoul-Fluor-based fluorescent bioprobe for a specific PTP. As shown in Fig. 1A, we introduced the *O*-phosphate moiety at the R^1 position to mimic *para*-substituted phenyl phosphate of phosphotyrosine and the acetyl moiety at the R^2 position to ensure good photophysical properties of bioprobes in an aqueous environment. Upon cleavage of the P–O bond by the enzymatic activity of a specific PTP, the liberated phenol moiety can perturb the electronic state of S*f*BP, which leads to the PeT-based reduction of the fluorescence signal. In order to maximize the on–off amplitude of the fluorescence signal, we can basify the resulting phenol with 1 N NaOH to form the phenoxide, which enhances the electron density at the R^1 position and further suppresses the emission signal of the bioprobe (Fig. 1B). Under this design hypothesis, we initiated the synthesis of S*f*BP from cinnamaldehyde derivative **1** prepared by a previously reported efficient 3-step synthesis (see Scheme 1). With an intramolecular 1,3-dipolar cycloaddition between olefin and azomethine ylide as a key step, five subsequent reactions allowed for the preparation of compound **6**.[8] After TBS deprotection, compound **6** was phosphorylated with diethyl phosphoroiodidate, generated *in situ* by the Arbuzov reaction of triethylphosphite with iodine,[12] to yield compound **7**. The hydrolysis of the resulting phosphodiester **7** with bromotrimethylsilane completed the preparation of S*f*BP in moderate yield (63.4% over 2 steps). For the enzyme kinetic study, the dephosphorylated form of S*f*BP (compound **8**) was also prepared *via* BOC deprotection of compound **6** with TFA (see ESI†).

The direct comparison of photophysical properties of S*f*BP and **8** showed that S*f*BP was a highly sensitive fluorescent probe for PTPs (Table 1 and Fig. 2). First, UV-Vis absorption and molar absorptivity of S*f*BP and **8** were consistent, both in phosphate buffered saline (PBS) and in 1 N NaOH, except for the 30-nm bathochromic shift of absorption maximum from S*f*BP to **8** in 1 N NaOH. In contrast, the existence of the phosphate



Scheme 1 Synthesis of Seoul-Fluor-based bioprobes for specific PTPs. *Reagents and conditions*: (a) *tert*-butyl-2-aminoethylcarbamate, AcOH, Na_2SO_4, DCM, rt; then $NaBH_4$, MeOH, 0 °C; (b) bromoacetyl bromide, TEA, DCM, −78 °C; (c) 4-acetylpyridine, DCM, 60 °C; then DBU, toluene; (d) DDQ; (e) HF/pyridine, THF; then TMSOMe; (f) I_2, DMAP, triethylphosphite, DCM, 0 °C; (g) TFA, DCM; (h) TMSBr.

Table 1 Photophysical properties of S*f*BP and **8** in PBS and 1N NaOH solution

Compound	Condition	λ_{abs} [a]	λ_{em} [b]	ε [c]	Φ [d]	$\varepsilon \times \Phi$	Fold [e]
S*f*BP	PBS	420	580	1.3×10^4	0.044	579.9	70
	1 N NaOH	417	580	1.3×10^4	0.058	778.4	93
8	PBS	420	580	7.4×10^3	0.019	140.1	17
	1 N NaOH	450	n/a	8.3×10^3	0.001	8.3	1

[a] Longest wavelength absorption maximum (nm). [b] Emission wavelength (nm) by the excitation at the absorption maximum. [c] Molar extinction coefficient (M^{-1} cm^{-1}) at absorption maximum. [d] Absolute quantum yield. [e] Fold difference of brightness ($\varepsilon \times \Phi$). Brightness of **8** in 1 N NaOH was normalized as 1 (see ESI).

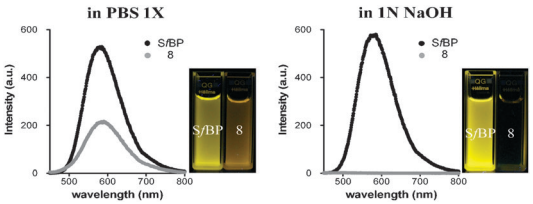


Fig. 2 Emission spectra and photographic images of S*f*BP (black) and **8** (gray) in PBS (left) and 1N NaOH (right), respectively.

group in S*f*BP caused dramatic effects on the fluorescence brightness ($\varepsilon \times \Phi$).[13] As shown in Table 1 and Fig. 2, S*f*BP in PBS was 4-fold brighter than **8**, and, surprisingly, S*f*BP was 93-fold brighter in the basic environment. Therefore, this result implies that the PeT-based fluorescence turn on–off of S*f*BP is a plausible mechanism for monitoring the activity of PTPs, and shows that S*f*BP is a highly sensitive fluorescent bioprobe useful for monitoring the activity of certain PTPs.

To test the specificity of S*f*BP, 65 different human PTPs, including 30 classical PTPs and 35 dual-specific PTPs, were individually expressed in *E. coli* and purified by affinity chromatography. Their activities were confirmed with 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP; see ESI†). As shown in Fig. 3, DiFMUP is a general substrate for PTPs and exhibits a

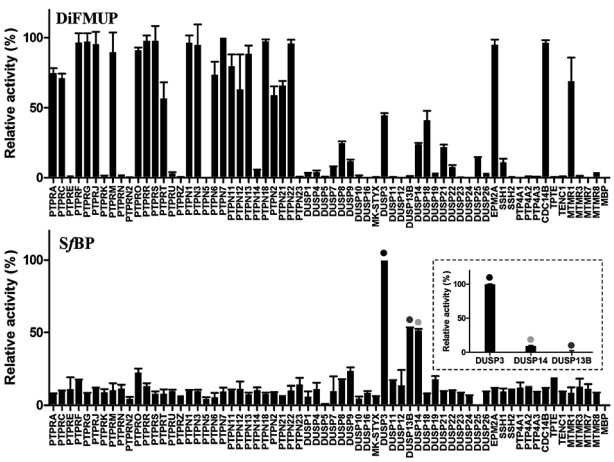


Fig. 3 Screening result of DiFMUP and S*f*BP against 65 different PTPs. Individual PTPs (1 μM) were incubated with either DiFMUP or S*f*BP at pH 8 to measure their selectivities. Inset data represent screening results of S*f*BP specificity to 3 different PTPs (DUSP3, DUSP14 and DUSP13B) at the lower enzyme concentration (0.1 μM).

Table 2 Kinetic parameters of DUSP3, DUSP13B, and DUSP14 for catalysis of S*f*BP and DiFMUP at pH 6

Enzyme	Substrate	Enzyme (nM)	K_m (μM)	k_{cat} (min^{-1})	k_{cat}/K_m (min^{-1} μM^{-1})
DUSP3	S*f*BP	10	34.5	159.36	4.62
(VHR)	DiFMUP	0.2	39.1	408.64	10.45
DUSP14	S*f*BP	100	18.2	6.53	0.36
	DiFMUP	1.0	48.5	60.54	1.25
DUSP13B	S*f*BP	2800	71.3	0.73	0.01
	DiFMUP	25	302.4	147.98	0.49

negligible selectivity for neither classical nor dual-specific PTPs. In contrast, we observed a good selectivity of S*f*BP toward VHR, also known as a dual-specific PTP-3 (DUSP3), in the initial screening and its excellent selectivity at the lower concentration (0.1 μM) of selected DUSPs and at pH 8.0 (see the inset of Fig. 3). Although catalytic activities of DUSPs were higher at pH 6, known as optimal pH for DUSPs,[14] than at pH 8, the selectivity was conserved at both pH values (see Fig. S5, ESI†). Since DUSP13B and DUSP14 showed meaningful catalytic activities to S*f*BP, especially at pH 6, their substrate specificity was determined using Lineweaver–Burk analysis and compared with that of DUSP3. Substrate specificity of DUSP3 to S*f*BP at pH 6 (k_{cat}/K_m = 4.62 min^{-1} μM^{-1}) was increased by 231 times from that at pH 8, and was 462- and 13-times higher than those of DUSP13B and DUSP14, respectively, at pH 6 (Table 2). Interestingly, the substrate specificity of S*f*BP toward three PTPs was dictated by the turnover number (k_{cat}) rather than binding affinity (K_m). While the K_m values are within 4-fold difference, the k_{cat} values showed up to 218-fold difference among three enzymes (Table 2), which indicates that S*f*BP binds to DUSP3 in proper orientation with proximity for its catalytic activity, but not to DUSP14 and DUSP13B.

Using *in silico* analysis, we proposed a plausible explanation of this selectivity of S*f*BP with a good docking score at the active site of VHR, which has a relatively shallow pocket. In contrast, the active sites of classical PTPs contain a deep and narrow pocket, where S*f*BP cannot be accommodated due to the presence of the bulky indolizine heterocycle at the *para* position of the phosphate group, thereby making itself a poor substrate for classical PTPs (see ESI†). Although the selectivity of S*f*BP toward VHR among 35 dual-specific PTPs is not yet fully addressed, this notable selectivity of S*f*BP toward VHR might be caused by the drug-like indolizine core skeleton,[17] which implies that pharmacophore-embedded fluorescent compounds[18] could function as powerful research tools for providing valuable information about players in the proteomic arena to elucidate complicated cellular processes. In fact, VHR has been recognized as a biomarker for various cancers including prostate[15] and cervical cancers.[16] Therefore, we envision that the VHR-specific fluorescent bioprobe, S*f*BP, can provide new insight into the design of potential therapeutics for prostate and cervical cancers.

In summary, we have developed a new fluorescent bioprobe that is selective for a specific PTP, VHR (DUSP3), among 30 classical and 35 dual-specific PTPs, using a PeT-based turn on–off mechanism upon dephosphorylation caused by the PTP activity. Due to its unparalleled selectivity, short detection time, over 90-fold signal enhancement, and the applicability to high throughput screening (HTS), S*f*BP-based assays could be a promising tool for the discovery of specific VHR inhibitors for the identification of therapeutic agents for VHR-related diseases, *e.g.*, prostate and cervical cancers. The X-ray structural analysis and development of an S*f*BP-based selective inhibitor for VHR are currently being investigated and will be reported in due course.

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Notes and references

1 (*a*) T. Hunter, *Cell*, 1995, **80**, 225–236; (*b*) N. K. Tonks, *Nat. Rev. Mol. Cell Biol.*, 2006, **7**, 833–846; (*c*) J. A. Ubersax and J. E. Ferrell Jr., *Nat. Rev. Mol. Cell Biol.*, 2007, **8**, 530–541.
2 Z. Zhang, *Curr. Opin. Chem. Biol.*, 2001, **5**, 416–423.
3 T. O. Johnson, J. Ermolieff and M. R. Jirousek, *Nat. Rev. Drug Discovery*, 2002, **1**, 696–709.
4 E. H. Fischer, H. Charbonneau and N. K. Tonks, *Science*, 1991, **253**, 401–406.
5 L. Tautz and T. Mustelin, *Methods*, 2007, **42**, 250–260.
6 (*a*) A. E. Stewart, S. Dowd, S. M. Keyse and N. Q. McDonald, *Nat. Struct. Biol.*, 1999, **6**, 174–181; (*b*) T. Yokota, Y. Nara, A. Kashima, K. Matsubara, S. Misawa, R. Kato and S. Sugio, *Proteins: Struct., Funct., Genet.*, 2007, **66**, 272–278.
7 (*a*) T.-I. Kim, H. J. Kang, G. Han, S. J. Chung and Y. Kim, *Chem. Commun.*, 2009, 5895–5897; (*b*) T.-I. Kim, M. S. Jeong, S. J. Chung and Y. Kim, *Chem.–Eur. J.*, 2010, **16**, 5297–5300.
8 (*a*) E. Kim, M. Koh, J. Ryu and S. B. Park, *J. Am. Chem. Soc.*, 2008, **130**, 12206–12207; (*b*) E. Kim, M. Koh, B. J. Lim and S. B. Park, *J. Am. Chem. Soc.*, 2011, **133**, 6642–6649.
9 (*a*) E. Kim, S. Lee and S. B. Park, *Chem. Commun.*, 2011, **47**, 7734–7736; (*b*) E. Kim, S. Lee and S. B. Park, *Chem. Commun.*, 2012, **48**, 2331–2333.
10 H. Kobayashi, M. Ogawa, R. Alford, P. L. Choyke and Y. Urano, *Chem. Rev.*, 2010, **110**, 2620–2640.
11 (*a*) J. Bermudez, C. S. Fake, G. F. Joiner, K. A. Joiner, F. D. King, W. D. Miner and G. J. Sanger, *J. Med. Chem.*, 1990, **33**, 1924–1929; (*b*) S. Hagishita, M. Yamada, K. Shirahase, T. Okada, Y. Murakami, Y. Ito, T. Matsuura, M. Wada, T. Kato, M. Ueno, Y. Chikazawa, K. Yamada, T. Ono, I. Teshirogi and M. Ohtani, *J. Med. Chem.*, 1996, **39**, 3636–3658.
12 A. Skowronska, M. Pakulski, J. Michalski, D. Cooper and S. Trippett, *Tetrahedron Lett.*, 1980, **21**, 321–322.
13 L. D. Lavis and R. T. Raines, *ACS Chem. Biol.*, 2008, **3**, 142–155.
14 S. Wu, S. Vossius, S. Rahmouni, A. V. Miletic, T. Vang, J. Vazquez-Rodriguez, F. Cerignoli, Y. Arimura, S. Williams, T. Hayes, M. Moutschen, S. Vasile, M. Pellecchia, T. Mustelin and L. Tautz, *J. Med. Chem.*, 2009, **52**, 6716–6723.
15 S. Rahmouni, F. Cerignoli, A. Alonso, T. Tsutji, R. Henkens, C. Zhu, C. Louis-dit-Sully, M. Moutschen, W. Jiang and T. Mustelin, *Nat. Cell Biol.*, 2006, **8**, 524–531.
16 R. Henkens, P. Delvenne, M. Arafa, M. Moutschen, M. Zeddou, L. Tautz, J. Boniver, T. Mustelin and S. Rahmouni, *BMC Cancer*, 2008, **8**, 147.
17 T. Weide, L. Arve, H. Prinz, H. Waldmann and H. Kessler, *Bioorg. Med. Chem. Lett.*, 2006, **16**, 59–63.
18 O. N. Burchak, L. Mugherli, M. Ostuni, J. J. Lacapère and M. Y. Balakirev, *J. Am. Chem. Soc.*, 2011, **133**, 10058–10061.