ChemComm

# Photoactivation of imatinib-antibody conjugate using low-energy visible light from Ru(II)-polypyridyl cages



| | |
|---|---|
| Journal: | *ChemComm* |
| Manuscript ID | CC-COM-02-2018-001348.R1 |
| Article Type: | Communication |
| | |

Journal Name

COMMUNICATION

# Photoactivation of imatinib-antibody conjugate using low-energy visible light from Ru(II)-polypyridyl cages

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Thomas N. Rohrabaugh, Jr.,[a] Ashley M. Rohrabaugh,[b] Jeremy J. Kodanko,[c]* Jessica K. White,[d]* and Claudia Turro[a]*

**Ru(II)-polypyridyl cages with sterically bulky bidentate ligands provide efficient photochemical release of the anticancer drug imatinib using low energy visible light, imparting spatiotemporal control over drug bioavailability. The light-activated drug release is maintained when the Ru(II) cage is covalently coupled to an antibody, which is expected to localize selectively on the tumor.**

Ru(II)-polypyridyl complexes are attractive candidates for a host of photochemically driven processes due to their rich light-absorbing properties and tunable photoreactivity.[1-6] In particular, such compounds have demonstrated versatility in the design of alternative molecules for photodynamic therapy (PDT) and photochemotherapy (PCT).[7-11] In traditional PDT, a relatively long-lived triplet excited state transfers energy to ground state $^3O_2$ to produce cytotoxic $^1O_2$ with high yield.[12, 13] PCT compounds that induce a cytotoxic or inhibitory effect on cancer cells following irradiation do not depend on the presence of $^3O_2$, a species that is typically present at low concentrations in solid tumors.[14, 15]

One class of PCT compounds photoreleases biologically active molecules from Ru(II)-polypyridyl protecting groups, or "cages." When the drug molecule is coordinated to the Ru(II) center through a functional group such as nitrile,[16-19] pyridyl,[20, 21] imidazole,[22-24] or amine,[25-28] its biological activity is inhibited. However, irradiation with visible light induces ligand exchange, whereby the active molecule is released and substituted by a solvent molecule. It is generally accepted that this process occurs by population of the thermally accessible triplet ligand field ($^3$LF) excited state(s), with Ru–(σ*) antibonding character.[29, 30] The population of this state in complexes with low energy triplet metal-to-ligand charge transfer ($^3$MLCT) excited states presents challenges due to the increased energy necessary to overcome the $^3$MLCT-$^3$LF state gap. We recently reported that the addition of steric bulk into the ligand structures in such complexes effectively lowers the $^3$LF state energy by distorting the pseudo-octahedral geometry around the metal center and decreasing the orbital overlap between the metal and the photolabile ligand.[31, 32] This stabilization of the $^3$LF state causes a dramatic enhancement of more than three orders of magnitude in the py ligand exchange quantum yields (Φ) for $[Ru(tpy)(L)(py)]^{2+}$, where L = 6,6′-dimethyl-2,2′-bipyridine ($Me_2$bpy) 2,2′-biquinoline (biq), or 3,6-dimethylbenzo[*i*]dipyrido[3,2-*a*:2′,3′-*c*]phenazine ($Me_2$dppn), compared to that of the $[Ru(tpy)(bpy)(py)]^{2+}$ analog (bpy = 2,2′-bipyridine) which lacks steric bulk.

The improvement in py dissociation efficiency in these $[Ru(tpy)(L)(py)]^{2+}$ complexes inspired us to use the $Ru^{II}$(tpy)(L) fragments as cages for the light-activated delivery of imatinib (STI-571), a tyrosine kinase inhibitor that is used in the treatment of nonresectable gastrointestinal stromal tumors (GISTs).[33, 34] More than half of GISTs begin in the stomach,[34] which is accessible by an endoscope, so the use of a light source to activate a drug at the GIST site is a promising early treatment option. Controlling the delivery of imatinib spatiotemporally by localized light activation may lessen the host of side effects often caused by the drug, including abdominal pain, decreased hemoglobin, nausea and vomiting, cardiac toxicity, and skin rashes and blistering.[35,36]

Herein we describe the synthesis, characterization, and photoinduced drug release in a series of $[Ru(tpy)(L)(imatinib)](PF_6)_2$ complexes, where L = $Me_2$bpy (**1**), biq (**2**), and $Me_2$dppn (**3**). The structures of the complexes are shown in Figure 1. Additionally, the complexes with L = $Me_2$bpy and biq were covalently coupled to the *c-kit*/CD117 antibody (Ab) to form **1-Ab** and **2-Ab**, respectively. The *c-kit*/CD117 enzyme is overexpressed in GISTs, therefore this work demonstrates the potential for such light-activated caged drug systems to be incorporated into antibody-drug conjugates to enhance localization of the drug at the tumor site(s).

[a] *Department of Chemistry and Biochemistry, The Ohio State University, Columbus, OH 43210, USA*
[b] *Department of Molecular Genetics, The Ohio State University, Columbus, OH 43210, USA*
[c] *Department of Chemistry, Wayne State University, Detroit, MI 48202, USA*
[d] *Department of Chemistry and Biochemistry, Ohio University, Athens, OH 45701, USA*

Electronic Supplementary Information (ESI) available: See DOI: 10.1039/x0xx00000x

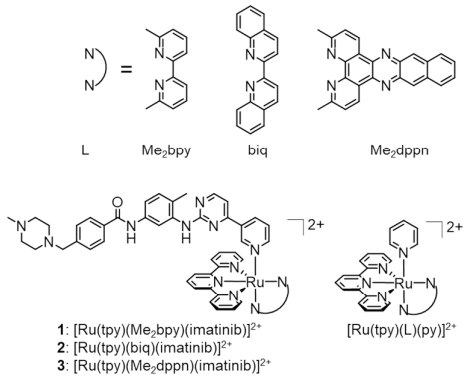


Fig 1. Structural representations of the bidentate ligands (L: $Me_2bpy$, biq, and $Me_2dppn$), **1**-**3**, and $[Ru(tpy)(L)(py)]^{2+}$.

The complexes were prepared by heating at reflux an ethanol solution of the previously reported $[Ru(tpy)(L)Cl]^{+}$ precursors with an excess of imatinib. Purification was achieved by precipitation of **1 – 3** upon addition of aqueous $NH_4PF_6$ followed by washing the solid with $Et_2O$. Analysis by $^1H$ NMR (Figures S1-S3) and ESI-MS (Figure S4) confirmed the identity and purity of the compounds. Details of the synthetic procedures, as well as NMR and MS data are presented in the Supporting Information.

The electronic absorption properties of **1 – 3** in $CH_3CN$ and $H_2O$ are similar to those reported for the $[Ru(tpy)(L)(py)](PF_6)_2$ analogs,[31, 32] indicating that the incorporation of the imatinib drug does not significantly impact the light absorbing properties of the Ru(II) caging chromophore. The overlaid spectra of the imatinib and the corresponding py complexes are presented in Figure S5. The visible region is dominated primarily by $^1$MLCT transitions. When the bidentate ligand, L, is $Me_2bpy$ (**1**) or $Me_2dppn$ (**3**), the lowest energy absorption band is assigned to overlapping Ru→tpy and Ru→L MLCT transitions with maxima at 472 nm (9330 $M^{-1}cm^{-1}$) and 487 nm (12,000 $M^{-1}cm^{-1}$), respectively. In the case where biq is the bidentate ligand (**2**), the lowest energy band is shifted to 528 nm (10,800 $M^{-1}cm^{-1}$) and is assigned as the Ru→biq MLCT transition, while the Ru→tpy MLCT transition occurs at higher energy, 440 nm (5170 $M^{-1}cm^{-1}$).

Irradiation of **1 – 3** with visible light in coordinating solvents results in the substitution of the monodentate imatinib ligand with a solvent molecule. In these reactions, the resulting free imatinib and the corresponding solvato complex are observed by $^1H$ NMR spectroscopy (Figure S6). This photoactivity is similar to that reported for the related $[Ru(tpy)(L)(py)](PF_6)_2$ complexes,[31,32] in which the pyridine ligand dissociates and is replaced by a solvent molecule. As shown in Figure 3, irradiation of **2** with red light ($\lambda_{irr} \geq$ 590 nm) results in a shift from 483 nm to 470 nm, consistent with the formation of $[Ru(tpy)(Me_2bpy)(OH_2)]^{2+}$ upon exchange of the imatinib ligand with a water solvent molecule. Similar spectral changes are observed for the photochemistry of the **2** and **3** (Figures S7 and S8) in water, which form the corresponding $[Ru(tpy)(L)(OH_2)]^{2+}$ complexes upon irradiation with red light. No spectral changes are observed in aqueous solution in the absence of light for at least one hour (Figures S9-S11).

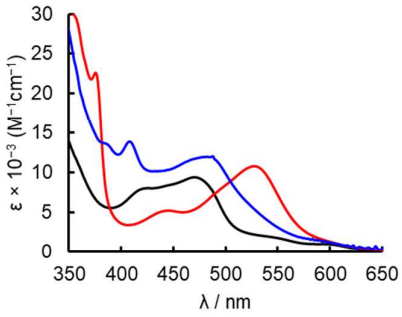


Fig 2. Overlaid electronic absorption spectra of **1** (black), **2** (red), and **3** (blue).

When the irradiation of **1 – 3** is conducted in $CH_3CN$, a blue shift in the MLCT band is observed upon formation of the $[Ru(tpy)(L)(CH_3CN)]^{2+}$ products (Figure S12-S14). The ligand exchange processes for **1**, **2**, and **3** in $CH_3CN$ occur with quantum yields ($\Phi_{500}$) of 0.25(2), 0.058(1), and 0.073(1), respectively ($\lambda_{irr}$ = 500 nm). These values are greater than those previously reported for the corresponding $[Ru(tpy)(L)(py)]^{2+}$ complexes, with $\Phi_{500}$ = 0.16(1), 0.033(1), and 0.053(1) when L = $Me_2bpy$, biq, and $Me_2dppn$, respectively.[31,32] The better efficiency for imatinib release compared to that of py may be due to the larger size of imatinib that may prevent it from easily recombining with the Ru(II) center once it rotates, such that the pyridyl substituent is no longer pointing toward the metal center. The higher efficiency could also be attributed to the weaker alkalinity of the coordinate nitrogen atom compared to that of py. The trend between the three caging molecules, however, remains consistent with that observed in the py complexes.[31,32]

In addition to the release of the drug imatinib, **3** sensitizes $^1O_2$ with $\Phi_\Delta$ = 0.57(7) which is similar to the value reported for $[Ru(tpy)(Me_2dppn)(py)^{2+}$, 0.69(9).[31] It is also important to note that following imatinib release, the photoproduct $[Ru(tpy)(Me_2dppn)(OH_2)]^{2+}$ remains active for $^1O_2$ generation with $\Phi$ = 0.22(2), showing that the Ru(II) fragment remains active for PDT after the drug is uncaged. Overall, the photochemical data suggest that the $Ru^{II}(tpy)(L)$ fragments can serve as cages with sterically bulky bidentate L ligands, making them promising molecular architectures for red-light activated drug release when the drug has an appropriate functional group for metal coordination.

To increase the potential functionality of these caged drug conjugates, the tpy ligand was substituted for tpy-COOH (2,2′:6′,2″-terpyridine-4′-carboxylic acid) and the [Ru(tpy-

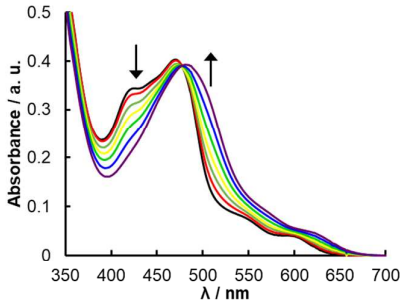


Fig 3. Changes in the electronic absorption spectroscopy of $[Ru(tpy)(Me_2bpy)(imatinib)](PF_6)_2$ in $H_2O$ (< 5% acetone) upon irradiation with $\lambda_{irr} \geq$ 590 nm for 0-60 min.

COOH)(L)(imatinib)]$^{2+}$ complexes were prepared with L = $Me_2$bpy (**1-COOH**) and biq (**2-COOH**). The carboxylic acid on the tpy ligand allows facile coupling to an antibody (Ab) that targets *c-kit*/CD117 and is overexpressed in GISTs.[37-39] The Ab was covalently coupled to the Ru(II)-imatinib complexes using a peptide coupling reaction between surface amine groups on the antibody and carboxylic acid-substituted tpy on **1-COOH** and **2-COOH**, which were synthesized in the same way as the analogous **1** and **2**. The carboxylic acid was converted to the NHS-ester by the reaction with NHS and DCC, and the [Ru(tpy-NHSester)(L)(imatinib)]$^{2+}$ was stirred in the dark at room temperature with the antibody in PBS buffer (pH = 7.5) for 1 h to produce the metal complex-antibody conjugates **1-Ab** and **2-Ab** (L = $Me_2$bpy and biq, respectively). Size exclusion chromatography was used to remove unreacted **1-COOH** and **2-COOH** from the samples. Detailed synthetic procedures are provided in the Supporting Information.

The electronic absorption spectra of the antibody-metal complex conjugate **1-Ab** as well as those of the Ab and **1** are shown in Fig. 4, showing that that the product spectrum exhibits features from both Ab alone ($\lambda_{max}$ = 280 nm) and unconjugated **1** ($\lambda_{max}$ = 480 nm). This finding clearly indicates that both the antibody and metal complex are present in the high molecular weight sample eluted in the size exclusion chromatography column. The electronic absorption spectrum for the **2-Ab** conjugate is presented in Figure S15. Based on the absorbance at 280 nm and at the lowest energy MLCT band, the samples of **1-Ab** and **2-Ab** were determined to have an average of 10 and 3 metal complexes, respectively, per Ab. These ratios are within or near an optimal range of drug loading previously reported for Ab-drug and Ab-dye conjugates.[40,41] To ensure that the metal complex is covalently bound to the Ab, a control experiment was performed in which the same coupling synthesis was performed while substituting only [Ru(tpy-NHSester)(biq)(imatinib)]$^{2+}$ for [Ru(tpy)(biq)(imatinib)]$^{2+}$. The latter molecule does not possess the NHS-ester group necessary for coupling with an amine on the surface of the Ab. Following the room temperature reaction for 1 h, the antibody sample that eluted from the column was colorless, and the absorption spectrum showed no absorption bands in the visible region, consistent with the covalent binding of the metal complex in **1-Ab** and **2-Ab** through the carboxylic acid functional group rather than through weaker, non-covalent interactions.

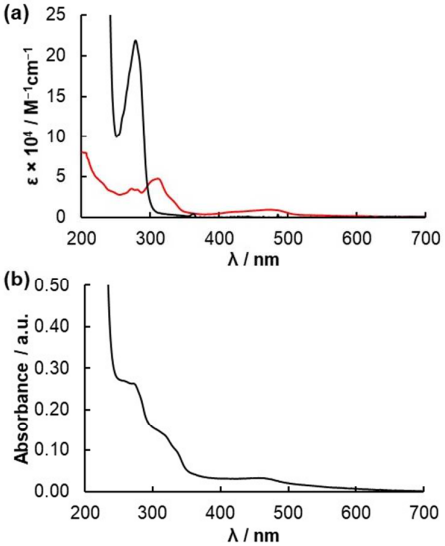


Fig 4. Electronic absorption spectra in PBS buffer for *c-kit* Ab (black) and **1** (red) (a) and the resulting **1-Ab** (b).

The photochemistry of **1-Ab** and **2-Ab** was investigated in PBS buffer (pH = 7.5) using red light ($\lambda_{irr} \geq 590$ nm). Irradiation of **1-Ab** results in a red shift in the $^1$MLCT transition from 460 nm to 510 nm (Figure 5), consistent with the substitution of the imatinib ligand with either $H_2O$ or $Cl^-$ from the buffer solution. A similar effect is observed upon the irradiation of **2-Ab**, with the $^1$MLCT transition shifting from 520 nm to 560 nm, as shown in Figure S16. These results demonstrate that conjugation of the [Ru(tpy)(L)(imatinib)]$^{2+}$ complexes to an Ab for directed drug delivery does not compromise the photoactivity of the metal complex cage. In addition, western blot analyses confirmed that the antibodie's selectivity is unaffected by the covalent modification of **1-Ab** and **2-Ab** (Figure S17).

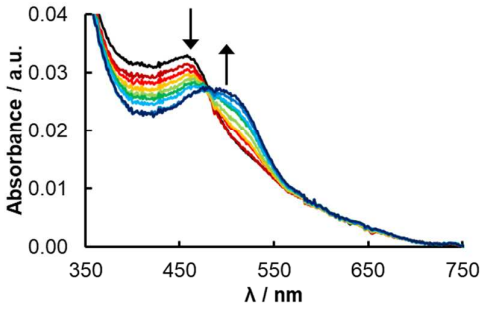


Fig 5. Changes in the electronic absorption spectroscopy of **1-Ab** in PBS buffer (pH = 7.5) upon irradiation with $\lambda_{irr} \geq 590$ nm for 0-90 min.

In the present work, we demonstrated that the drug imatinib can be caged using appropriate Ru(II) complexes with steric bulk, **1** – **3**, showing its release from the metal complex coordination sphere upon irradiation with low energy visible light. When the ancillary ligand is $Me_2$dppn (**3**), further irradiation of the complex following release of the drug sensitizes the production of $^1O_2$, giving this molecule dual therapeutic activity. The Ru-caged imatinib complexes were also covalently coupled to an antibody that targets the *c-kit* proto-oncogene that is overexpressed in gastrointestinal stromal tumors and serves as a selective homing site for the caged drug to target imatinib's activity. This antibody-drug conjugate is expected to enhance the photoactivated delivery of the drug to the tumor site. Work is ongoing to establish the

light-activated *in vitro* and *in vivo* activity of the new complexes and antibody-drug conjugates.

The authors thank the National Institutes of Health (EB 016072) and the National Science Foundation (CHE-1465067) for their generous support of this work. The authors would also like to thank Prof. Amanda Simcox for her assistance with the western blot measurements.

## Conflicts of interest

There are no conflicts to declare.

## Notes and references

1. C. K. Prier, D. A. Rankic and D. W. C. MacMillan, *Chem. Rev.*, 2013, **113**, 5322-5363.
2. N. Serpone, E. Pelizzetti and M. Gratzel, *Coord. Chem. Rev.*, 1985, **64**, 225-245.
3. D. W. Thompson, A. Ito and T. J. Meyer, *Pure and Applied Chem.*, 2013, **85**, 1257.
4. L. Hammarström, *Acc. Chem. Res.*, 2015, **48**, 840-850.
5. K. K.-W. Lo and S. P.-Y. Li, *RSC Adv.*, 2014, **4**, 10560-10585.
6. V. Balzani, G. Bergamini and P. Ceroni, *Coord. Chem. Rev.* 2008, **252**, 2456-2469.
7. G. Shi, S. Monro, R. Hennigar, J. Colpitts, J. Fong, K. Kasimova, H. Yin, R. DeCoste, C. Spencer and L. Chamberlain, *Coord. Chem. Rev.*, 2015, **282**, 127-138.
8. C. Mari, V. Pierroz, S. Ferrari and G. Gasser, *Chem. Sci.*, 2015, **6**, 2660-2686.
9. J. D. Knoll and C. Turro, *Coord. Chem. Rev.*, 2015, **282**, 110-126.
10. J. D. Knoll, B. A. Albani and C. Turro, *Acc. Chem. Res.*, 2015, **48**, 2280-2287.
11. N. J. Farrer, L. Salassa and P. J. Sadler, *Dalton Trans.*, 2009, 10690-10701.
12. K. Szaciłowski, W. Macyk, A. Drzewiecka-Matuszek, M. Brindell and G. Stochel, *Chem. Rev.*, 2005, **105**, 2647-2694.
13. I. J. Macdonald and T. J. Dougherty, *J. Porphyrins Phthalocyanines*, 2001, **5**, 105-129.
14. L. Wyld, M. Reed and N. Brown, *British J. Cancer*, 2001, **84**, 1384-1386.
15. C. Tanielian, C. Schweitzer, R. Mechin and C. Wolff, *Free Radical Biol. Med.*, 2001, **30**, 208-212.
16. M. A. Sgambellone, A. David, R. N. Garner, K. R. Dunbar and C. Turro, *J. Am. Chem. Soc.*, 2013, **135**, 11274-11282.
17. T. Respondek, R. Sharma, M. K. Herroon, R. N. Garner, J. D. Knoll, E. Cueny, C. Turro, I. Podgorski and J. J. Kodanko, *ChemMedChem*, 2014, **9**, 1306-1315.
18. T. Respondek, R. N. Garner, M. K. Herroon, I. Podgorski, C. Turro and J. J. Kodanko, *J. Am. Chem. Soc.*, 2011, **133**, 17164-17167.
19. R. N. Garner, J. C. Gallucci, K. R. Dunbar and C. Turro, *Inorg. Chem.*, 2011, **50**, 9213-9215.
20. (a) A. Li, R. Yadav, J. K. White, M. K. Herroon, B. P. Callahan, I. Podgorski, C. Turro, E. E. Scott and J. J. Kodanko, *Chem. Commun.*, 2017, **53**, 3673-3676. (b) M. Huisman, J. K. White, V. G. Lewalski, I. Podgorski, C. Turro and J. J. Kodanko, *Che. Commun.*, 2016, **52**, 12590-12593.
21. N. A. Smith, P. Zhang, S. E. Greenough, M. D. Horbury, G. J. Clarkson, D. McFeely, A. Habtemariam, L. Salassa, V. G. Stavros, C. G. Dowson and P. J. Sadler, *Chem. Sci.*, 2017, **8**, 395-404.
22. J. Mosquera, M. I. Sanchez, J. L. Mascarenas and M. Eugenio Vazquez, *Chem. Commun.*, 2015, **51**, 5501-5504.
23. N. Karaoun and A. K. Renfrew, *Chem. Commun.*, 2015, **51**, 14038-14041.
24. H. Chan, J. B. Ghrayche, J. Wei and A. K. Renfrew, *Eur. J. Inorg. Chem.*, 2017, **2017**, 1679-1686.
25. L. Zayat, M. G. Noval, J. Campi, C. I. Calero, D. J. Calvo and R. Etchenique, *ChemBioChem*, 2007, **8**, 2035-2038.
26. O. Filevich, M. Salierno and R. Etchenique, *J. Inorg. Biochem.*, 2010, **104**, 1248-1251.
27. O. Filevich and R. Etchenique, *Photochem. Photobiol. Sci.*, 2013, **12**, 1565-1570.
28. R. Araya, V. Andino-Pavlovsky, R. Yuste and R. Etchenique, *ACS Chem. Neurosci.*, 2013, **4**, 1163-1167.
29. E. Tfouni, *Coord. Chem. Rev.*, 2000, **196**, 281-305.
30. G. Malouf and P. C. Ford, *J. Am. Chem. Soc.*, 1977, **99**, 7213-7221.
31. J. D. Knoll, B. A. Albani and C. Turro, *Chem. Commun.*, 2015, **51**, 8777-8780.
32. J. D. Knoll, B. A. Albani, C. B. Durr and C. Turro, *J. Phys. Chem. A*, 2014, **118**, 10603-10610.
33. M. C. Heinrich, D. J. Griffith, B. J. Druker, C. L. Wait, K. A. Ott and A. J. Zigler, *Blood*, 2000, **96**, 925-932.
34. U. De Giorgi and J. Verweij, *Mol. Cancer Therapeutics*, 2005, **4**, 495-501.
35. N. Scheinfeld, *J. Drugs Dermatology*, 2006, **5**, 228-231.
36. H. Joensuu, J. C. Trent and P. Reichardt, *Cancer Treatment Rev.*, 2011, **37**, 75-88.
37. M. Sarlomo-Rikala, A. J. Kovatich, A. Barusevicius and M. Miettinen, *Modern Pathology*, 1998, **11**, 728-734.
38. B. P. Rubin, S. Singer, C. Tsao, A. Duensing, M. L. Lux, R. Ruiz, M. K. Hibbard, C.-J. Chen, S. Xiao, D. A. Tuveson, G. D. Demetri, C. D. M. Fletcher and J. A. Fletcher, *Cancer Res.*, 2001, **61**, 8118-8121.
39. M. C. de Silva and R. Reid, *Pathol. Oncol. Res.* 2003, **9**, 13-19.
40. K. J. Hamblett, P. D. Senter, D. F. Chace, M. M. C. Sun, J. Lenox, C. G. Cerveny, K. M. Kissler, S. X. Bernhardt, A. K. Kopcha, R. F. Zabinski, D. L. Meyer and J. A. Francisco, *Clin. Cancer Res.*, 2004, **10**, 7063-7070.
41. S. Vira, E. Mekhedov, G. Humphrey and P. S. Blank, *Anal. Biochem.*, 2010, **402**, 146-150.