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Cite this: DOI: 10.1039/c0xx00000x

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ARTICLE TYPE

# Photodegradation of amyloid $\beta$ and reduction of its cytotoxicity to PC12 cells using porphyrin derivatives

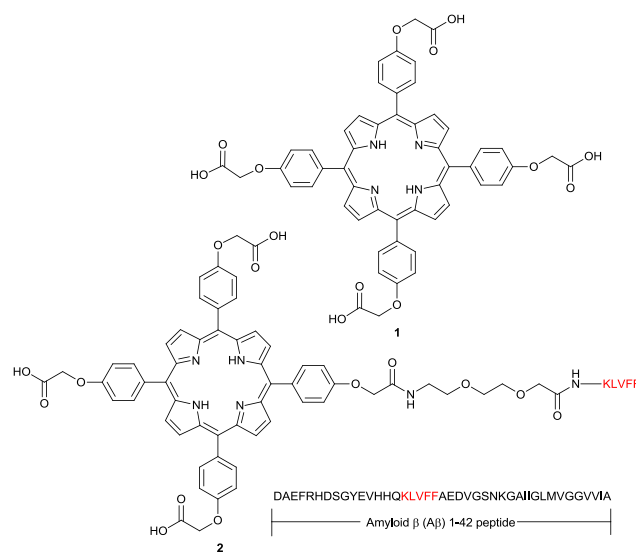
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Received (in XXX, XXX) Xth XXXXXXXXXX 20XX, Accepted Xth XXXXXXXXXX 20XX

DOI: 10.1039/b000000x

A purpose-designed porphyrin-peptide hybrid effectively degraded amyloid  $\beta$  monomer and oligomers associated with Alzheimer's disease. Degradation was achieved using light irradiation in the absence of any additives and under neutral conditions. Moreover, the hybrid effectively neutralized the cytotoxicity of amyloid  $\beta$  in PC12 cells upon photoirradiation.

Alzheimer's disease (AD)<sup>1</sup> is a progressive neurodegenerative disorder and is the most common form of dementia in humans. It is characterized by neuronal loss and the presence of large numbers of senile plaques, consisting of fibrillar aggregates of 40- and 42-residue amyloid  $\beta$  (A $\beta$ ) peptides, in the brain.<sup>2</sup> In recent studies, it was suggested that soluble oligomers of A $\beta_{42}$  are responsible for synaptic dysfunction in the brains of patients with AD and are the key intermediate neurotoxic species in the pathology of AD.<sup>3</sup> In addition, it was revealed that the hydrophobic KLVFF region of A $\beta_{42}$  is an important element responsible for the aggregation of A $\beta_{42}$  (Fig. 1).<sup>4</sup> One way of suppressing A $\beta_{42}$  assembly in the brain is through small molecules with a high affinity for<sup>5</sup> or ability to degrade<sup>6</sup> A $\beta_{42}$ . A major effort should be directed toward inhibiting amyloid formation at very early stages of the disease. In addition, using the degradation approach, even if the inhibitor diffuses away, the target A $\beta_{42}$  is degraded and remains inactive, resulting in irreversible inhibition of A $\beta_{42}$  function and an apparent increase in potency. In this context, we recently disclosed that designed and synthesized fullerene derivatives effectively inhibited A $\beta_{42}$  aggregation and degraded A $\beta_{42}$  under photoirradiation conditions.<sup>7</sup> Following our reports, Qu et al. reported the photodegradation of  $\beta$ -sheet amyloid using inorganic molecules, polyoxometalates, as photocatalysts.<sup>8</sup> On the other hand, we found that certain porphyrin derivatives could degrade proteins by emitting reactive oxygen species under photoirradiation conditions.<sup>9</sup> In addition, certain porphyrins were independently found to inhibit A $\beta_{40}$  filament formation.<sup>10</sup> Based on these findings, we hypothesized that a porphyrin itself or a porphyrin hybrid possessing a KLVFF structure as a key segment for A $\beta_{40}$  aggregation would effectively degrade A $\beta_{42}$  under photoirradiation conditions, and could be used to neutralize the cytotoxicity of A $\beta_{42}$  in neuron cells. In this communication, we



**Fig. 1** Chemical structures of porphyrin derivative **1**, porphyrin-peptide (KLVFF) hybrid **2**, and A $\beta_{42}$  peptide.

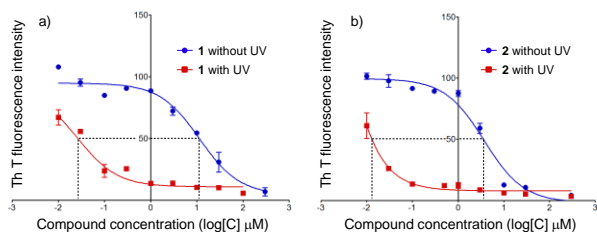
present the molecular design, chemical synthesis and evaluation of a novel and artificial light-activatable porphyrin-peptide (KLVFF) hybrid molecule that can effectively degrade A $\beta_{42}$  and neutralize its cytotoxicity under photoirradiation.

To investigate our hypothesis, we utilized porphyrin derivative **1** as a protein photodegrading agent and designed porphyrin-peptide (KLVFF) hybrid **2** with an appropriate spacer between **1** and the peptide (KLVFF) (Fig. 1). After chemical synthesis of the designed hybrid **2** using solid-phase synthesis (see ESI: Scheme S1), we first examined the binding abilities of **1** and **2** with A $\beta_{42}$  by observation of the inhibitory effect using a thioflavin T (Th T) fluorescence assay.<sup>11</sup> The inhibitory concentration (IC<sub>50</sub>) was obtained by measuring the Th T fluorescence intensity as a function of the concentration of **1** or **2**. Figs. 2a and 2b show the plots obtained, which give IC<sub>50</sub> values of 11.0 and 3.56  $\mu$ M for **1** and **2**, respectively. These results indicated that both **1** and **2** had high affinity for A $\beta_{42}$ , and that the affinity of the porphyrin-peptide hybrid **2** was 3 times stronger than that of **1**. Next, the difference in the A $\beta_{42}$  aggregation-inhibitory abilities of **1** and **2** with and without photoirradiation (365 nm, 100 W) was confirmed by a Th T fluorescence assay. It was found that the IC<sub>50</sub> values of **1** and **2** were 26.9 and 12.9 nM, respectively, with photoirradiation. These results clearly indicate that **1** and **2** inhibit

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† Electronic Supplementary Information (ESI) available: See DOI: 10.1039/b000000x/

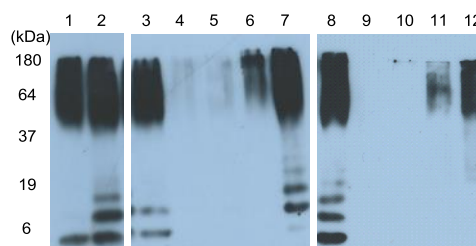
$A\beta_{42}$  aggregation much more efficiently under photoirradiation. The inhibitory ability of **2** was 2 times higher than that of **1** under photoirradiation. Furthermore, it is noteworthy that the inhibitory ability of **2** was 45 times higher than that of the previously reported fullerene derivative<sup>7</sup> under photoirradiation.



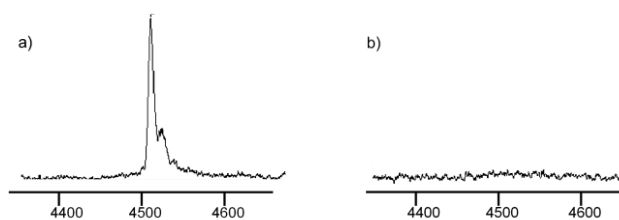
**Fig. 2** Variation in Th T fluorescence intensity as a function of the concentration of **1** or **2** with and without photoirradiation (365 nm, 100 W, 10 cm, 2 h).  $A\beta_{42}$  (40  $\mu\text{M}$ ) was incubated with each compound (300–0.01  $\mu\text{M}$ ) in 10% DMF/Tris-HCl buffer (pH 8.0, 20 mM) at 37  $^{\circ}\text{C}$  for 24 h, then Th T (6.67  $\mu\text{M}$ ) was added to each sample. Fluorescence was measured using a Safire (TECAN) microplate reader using excitation and emission wavelengths of 430 nm and 491 nm, respectively. Data were analyzed using GraphPad Prism to obtain  $\text{IC}_{50}$  values using log (compound) vs. normalized response-variable slope. a) Dose-response curves showing fractional binding of Th T to  $A\beta_{42}$  fibrils in the presence of **1** with (red line) or without (blue line) photoirradiation. b) Dose-response curves showing fractional binding of Th T to  $A\beta_{42}$  fibrils in the presence of **2** with (red line) or without (blue line) photoirradiation.

With these favourable results in hand, we next examined the  $A\beta_{42}$  photodegradation ability of **1** and **2** against a mixture of  $A\beta_{42}$  monomer and oligomers<sup>12</sup> upon photoirradiation. The results of the photodegradation experiment, which was monitored by SDS-PAGE and immunoblotting with monoclonal antibody 6E10,<sup>12</sup> are shown in Fig. 3. Comparison of lanes 2, 3 and 8 with lane 1 shows that neither photoirradiation of  $A\beta_{42}$  monomer and oligomers in the absence of **1** or **2** (lanes 2 in Fig. 3), or treatment of  $A\beta_{42}$  monomer and oligomers with **1** or **2** but without photoirradiation (lanes 3 and 8 in Fig. 3), resulted in a change in the SDS-PAGE profile. In contrast, lanes 4–6 and 9–12 show the disappearance of the SDS-PAGE bands corresponding to both  $A\beta_{42}$  monomer and oligomers after exposure to **1** or **2** and photoirradiation. This indicates that degradation of  $A\beta_{42}$  monomer and oligomers took place. These results show that porphyrin derivatives **1** and **2** are capable of degrading not only the  $A\beta_{42}$  monomer but also its oligomers upon irradiation with long-wavelength UV light under neutral conditions and in the absence of other additives, and that the photodegrading ability of **2** is higher than that of **1**. In all cases, it was confirmed that  $A\beta_{42}$  monomer was more effectively degraded than  $A\beta_{42}$  oligomers by the porphyrin derivatives. Because the degradation of  $A\beta_{42}$  monomer and oligomers by the porphyrin derivatives did not take place in the absence of light, it was confirmed that UV light functioned as a trigger to initiate degradation by the porphyrin derivatives.

These photodegradation results were also supported by analysis using MALDI-TOF MS, as shown in Figure 4. Thus, after the incubation of  $A\beta_{42}$  with **2** under photoirradiation, the MS peak corresponding to  $A\beta_{42}$  disappeared. No MS peaks corresponding to the degradation fragments could be detected due to the randomness of the degradation,<sup>7</sup> leading to many fragments present in very small amounts.



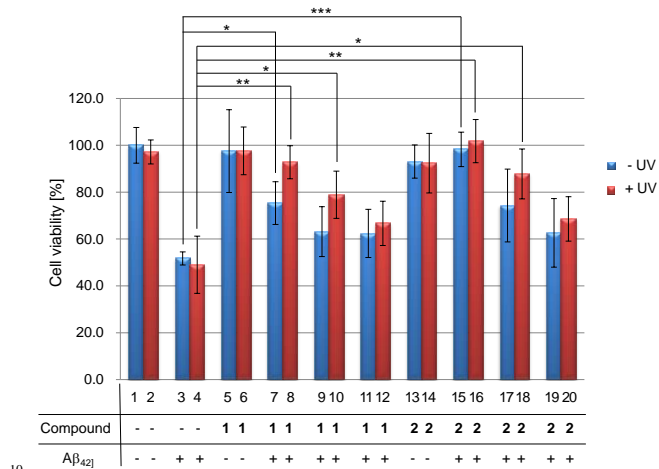
**Fig. 3.** Photodegradation of  $A\beta_{42}$  by **1** or **2**. A mixture of  $A\beta_{42}$  monomer and oligomers (5.0  $\mu\text{M}$ ) was incubated with each compound in 10% DMF/Tris-HCl buffer (pH 8.0, 20 mM) at 25  $^{\circ}\text{C}$  for 2 h with or without photoirradiation using a UV lamp (365 nm, 100 W) placed 10 cm from the sample. Each sample was analyzed using tricine-SDS-PAGE and immunoblotting with monoclonal antibody 6E10. Lane 1:  $A\beta_{42}$  alone; lane 2:  $A\beta_{42}$  with photoirradiation; lane 3:  $A\beta_{42}$  + **1** (50  $\mu\text{M}$ ) without photoirradiation; lanes 4–7:  $A\beta_{42}$  + **1** (at a concentration of 50, 15, 5.0 and 1.5  $\mu\text{M}$ , respectively) with photoirradiation; lane 8:  $A\beta_{42}$  + **2** (50  $\mu\text{M}$ ) without photoirradiation; lanes 9–12:  $A\beta_{42}$  + **2** (at a concentration of 50, 15, 5.0 and 1.5  $\mu\text{M}$ , respectively) with photoirradiation.



**Fig. 4.** MALDI-TOF MS profile of  $A\beta_{42}$  peptide obtained by photodegradation using peptide-porphyrin hybrid **2**.  $A\beta_{42}$  peptide (5.0  $\mu\text{M}$ ) was incubated with **2** (50  $\mu\text{M}$ ) in 10% DMF/Tris-HCl buffer (pH 8.0, 20 mM) at 25 $^{\circ}\text{C}$  for 2 h with b) or without a) photoirradiation using a UV lamp (365 nm, 100 W) placed 10 cm from the mixture. The resulting products were analyzed by MALDI-TOF MS (matrix: 3,5-dimethoxy-4-hydroxycinnamic acid). A peak was observed at  $m/z$  4516.2, indicating  $A\beta_{42}$  peptide in a); this peak was not observed in b).

Based on these results, we then examined the inhibition effect of porphyrin derivatives **1** and **2** on  $A\beta$ -mediated cytotoxicity in neuron-like cells with and without photoirradiation using rat pheochromocytoma (PC12) cells<sup>13</sup> and the MTT assay. PC12 cells were chosen for assessing toxicity because their sensitivity to the toxic effects of  $A\beta_{42}$  can be measured using MTT.<sup>14</sup> The inhibitors were mixed with 0.5  $\mu\text{M}$   $A\beta_{42}$  oligomer at concentrations of 0.3, 0.03 and 0.003  $\mu\text{M}$ , and the solutions were added to the cells. After pre-incubation with or without photoirradiation for 15 min, cell viability was determined after 24 h using the MTT assay. The results are summarized in Fig. 5. In the absence of photoirradiation, the survival rate of the control cells (cells incubated with only 0.5  $\mu\text{M}$   $A\beta_{42}$  oligomer) was approximately 50% (lane 3 in Fig. 5). It was found that porphyrin derivatives **1** and **2** showed no cytotoxicity (lanes 5 and 13, respectively, in Fig. 5), and reduced cell toxicity in a dose-dependent manner due to their aggregation inhibition of  $A\beta_{42}$  (lanes 7, 9, 11 and 15, 17, 19, respectively, in Fig. 5). Under conditions of photoirradiation, it was confirmed that photoirradiation itself had no effect on cell viability in the presence or absence of **1** or **2** (lane 2 in Fig. 5), and that **1** and **2** showed no cytotoxicity even with photoirradiation (lanes 6 and 14, respectively, in Fig. 5). Furthermore, it is noteworthy that **1** and **2** significantly countered  $A\beta_{42}$ -mediated cytotoxicity in PC12 cells with photoirradiation in

a dose-response manner (lanes 8, 10, 12 and 16, 18, 20, respectively, in Fig. 5), and that the inhibition ability of **1** and **2** with photoirradiation was higher than without photoirradiation. In addition, the inhibition ability of **2** was higher than that of **1** and much higher (~30 times) than the previously reported fullerene derivative.<sup>7</sup> Importantly, these results strongly suggest that the photodegradation of A $\beta$ <sub>42</sub> by **1** and **2** took place in PC12 cells in a highly target-selective manner, allowing PC12 cells to survive even in the presence of toxic A $\beta$ <sub>42</sub>.



**Fig. 5.** Cell culture assay. PC12 cells were seeded into 96-well plates ( $4 \times 10^3$  per well). Cells were pre-incubated with A $\beta$ <sub>42</sub> oligomer (0.5  $\mu$ M) and **1** or **2** for 15 minutes at 25  $^{\circ}$ C with or without photoirradiation using a UV lamp (365 nm, 30 W) placed 15 cm from the sample; the samples were then further incubated at 37  $^{\circ}$ C for 24 h. Cell viability was determined using the MTT viability assay. a) Lanes 1 and 2: PC12 alone without and with photoirradiation, respectively; lanes 3 and 4: PC12 + A $\beta$ <sub>42</sub> oligomer (0.5  $\mu$ M) without and with photoirradiation, respectively; lanes 5 and 6: PC12 + **1** (1.0  $\mu$ M) without and with photoirradiation, respectively; lanes 7 and 8: PC12 + **1** (0.3  $\mu$ M) + A $\beta$ <sub>42</sub> oligomer (0.5  $\mu$ M) without and with photoirradiation, respectively; lanes 9 and 10: PC12 + **1** (0.03  $\mu$ M) + A $\beta$ <sub>42</sub> oligomer (0.5  $\mu$ M) without and with photoirradiation, respectively; lanes 11 and 12: PC12 + **1** (0.003  $\mu$ M) + A $\beta$ <sub>42</sub> oligomer (0.5  $\mu$ M) without and with photoirradiation, respectively; lanes 13 and 14: PC12 + **2** (1.0  $\mu$ M) without and with photoirradiation, respectively; lanes 15 and 16: PC12 + **2** (0.3  $\mu$ M) + A $\beta$ <sub>42</sub> oligomer (0.5  $\mu$ M) without and with photoirradiation, respectively; lanes 17 and 18: PC12 + **2** (0.03  $\mu$ M) + A $\beta$ <sub>42</sub> oligomer (0.5  $\mu$ M) without and with photoirradiation, respectively; lanes 19 and 20: PC12 + **2** (0.003  $\mu$ M) + A $\beta$ <sub>42</sub> oligomer (0.5  $\mu$ M) without and with photoirradiation, respectively. Bar graph showed the means and standard derivations from at least three independent and reproducible experiments. \* $p$  < 0.05, \*\* $p$  < 0.01, \*\*\* $p$  < 0.001.

In conclusion, we have developed a novel method for the inhibition of the cytotoxic effects of A $\beta$ <sub>42</sub> in neuron-like PC12 cells using porphyrin derivatives in conjunction with photoirradiation to degrade A $\beta$ <sub>42</sub> monomer and oligomers. Pin-point photoirradiation permits high-resolution control over a defined area and low interference with normal biological functions. The results presented here will aid in the molecular design of artificial protein photodegradation agents which will, in turn, help provide a means of controlling the specific functions of certain proteins.

This research was supported in part by Scientific Research (B) (No. 23310153 and 26282212) and Scientific Research on Innovative Areas “Chemical Biology of Natural Products” (No. 24102528 and 26102738) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT).

## Notes and references

- J. Hardy and G. A. Higgins, *Science*, 1992, **256**, 184; C. Haass and B. De Strooper, *Science*, 1999, **286**, 916; D. J. Selkoe, *Physiol. Rev.*, 2001, **81**, 741; J. Hardy and D. J. Selkoe, *Science*, 2002, **297**, 353.
- D. J. Selkoe, *Nature Suppl.*, 1999, **399**, A23-A31; K. Sambamurti, N. H. Greig and D. K. Lahiri, *J. Neuromol. Med.*, 2002, **1**, 1.
- S. A. Gravina, L. B. Ho, C. B. Eckman, K. E. Long, L. Otvos, Jr., L. H. Younkin, N. Suzuki and S. G. Younkin, *J. Biol. Chem.*, 1995, **270**, 7013; M. P. Lambert, A. K. Barlow, B. A. Chromy, C. Edwards, R. Freed, M. Liosatos, T. E. Morgan, I. Rozovsky, B. Trommer, K. L. Viola, P. Wals, C. Zhang, C. E. Finch, G. A. Kraft and W. Klein, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 6448; O. M. El-Agnaf, D. S. Mahil, B. P. Patel and B. M. Austen, *Biochem. Biophys. Res. Commun.*, 2000, **273**, 1003; D. M. Walsh, I. Klyubin, J. V. Fadeeva, M. J. Rowan and D. J. Selkoe, *Biochem. Soc. Trans.*, 2002, **30**, 552; H. J. Kim, S. C. Chae, D. K. Lee, B. Chromy, S. C. Lee, Y. C. Park, W. L. Llein, G. A. Krafft and S. T. Hong, *FASEB J.*, 2003, **17**, 118; J. P. Cleary, D. M. Walsh, J. J. Hofmeister, G. M. Shankar, M. A. Kuskowski, D. J. Selkoe and K. H. Ashe, *Nat. Neurosci.*, 2004, **8**, 79; S. Li, S. Hong, N. E. Shephardson, D. M. Walsh, G. M. Shankar and D. Selkoe, *Neuron*, 2009, **62**, 788.
- N. Kokkoni, K. Stott, H. Amijee, J. M. Mason, *Biochemistry*, 2006, **32**, 9906; J. E. Kim and M. Lee, *Biochem. Biophys. Res. Commun.*, 2003, **303**, 576; L. O. Tjernberg, J. Näslund, F. Lindqvist, J. Johansson, A. R. Karlström, J. Thyberg, L. Terenius, and C. Nordstedt, *J. Biol. Chem.*, 1996, **271**, 8545.
- S. J. Wood, L. MacKenzie, B. Malceff, M. R. Hurlle and R. Wetzel, *J. Biol. Chem.*, 1996, **271**, 4086; D. R. Howlett, A. E. Perry, F. Godfrey, J. E. Swatton, K. H. Jennings, C. Spitzfaden, H. Wadsworth, S. J. Wood and R. E. Markwell, *Biochem. J.*, 1999, **340**, 283; D. R. Howlett, A. R. George, D. E. Owen, R. V. Ward and R. E. Markwell, *Biochem. J.*, 1999, **343**, 419; D. Allsop, G. Gibson, I. K. Martin, S. Moore, S. Turnbull and L. J. Twyman, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 255; F. G. Felice, J. Houzel, J. Garcia-Abreu, P. R. Louzada, Jr., R. C. Afonso, M. N. Meirelles, R. Lent, V. M. Neto and S. T. Ferreira, *FASEB J.*, 2001, **15**, 1297; F. Yang, G. P. Lim, A. N. Begum, O. J. Ubeda, M. R. Simmons, S. S. Ambegaokar, P. Chen, R. Kaye, C. G. Glabe, S. A. Frautschy and G. M. Cole, *J. Biol. Chem.*, 2005, **280**, 5892; T. Cohen, A. Frydman-Marom, M. Rechter and E. Gazit, *Biochemistry*, 2006, **45**, 4727; N. Kokkoni, K. Stott, H. Amijee, J. M. Mason and A. J. Doig, *Biochemistry*, 2006, **45**, 9906; H. Amijee, J. Madine, D. A. Middleton and A. D. Doig, *Biochem. Soc. Trans.*, 2009, **37**, 692.
- J. Suh, S. H. Yoo, M. G. Kim, K. Jeong, J. Y. Ahn, M. Kim, P. S. Chae, T. Y. Lee, J. Lee, J. Lee, Y. A. Jang and E. H. Ko, *Angew. Chem. Int. Ed.*, 2007, **46**, 7064.
- Y. Ishida, S. Tanimoto, D. Takahashi and K. Toshima, *Med. Chem. Commun.*, 2010, **1**, 212; Y. Ishida, T. Fujii, K. Oka, D. Takahashi and K. Toshima, *Chem. Asian J.*, 2011, **6**, 2312.
- M. Li, C. Xu, J. Ren, E. Wang and X. Qu, *Chem. Commun.*, 2013, **49**, 11394.
- S. Tanimoto, S. Matsumura and K. Toshima, *Chem. Commun.*, 2008, 3678; A. Okochi, S. Tanimoto, D. Takahashi, K. Toshima, *Chem. Commun.*, 2013, **49**, 6027.
- M. Masuda, N. Suzuki, S. Taniguchi, T. Oikawa, T. Nonaka, T. Iwatsubo, S. Hisanaga, M. Goedert and M. Hasegawa, *Biochemistry*, 2006, **45**, 6085.
- H. LeVine, III, *Protein Sci.*, 1993, **2**, 404.
- V. Rangachari, B. D. Moore, D. K. Reed, L. K. Sonoda, A. W. Bridges, E. Conboy, D. Hartigan and T. Rosenberry, *Biochemistry*, 2007, **46**, 12451.
- M. S. Shearman, C. I. Ragan and L. L. Iversen, *Proc. Nat. Acad. Sci. USA*, 1994, **91**, 1470.
- M. B. Hansen, S. E. Nielsen and K. Berg, *J. Immunol. Methods*, 1989, **119**, 203-210.