Photolithographic synthesis of cyclic peptide arrays using a differential deprotection strategy†

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We report here a strategy for the photolithographic synthesis of diverse, spatially addressable arrays of cyclic peptides which employs a differential deprotection strategy for the combinatorial addition of side chains to a pre-fabricated cyclic core.

There is considerable interest in the creation of small molecule microarrays as a platform with which to screen combinatorial libraries for protein ligands.[1] Most such arrays have been created using mechanical spotting,[2,3] but arrays with far more features can be created using photolithographic synthesis techniques.[4–7] We have recently developed chemistry that allows dense microarrays of peptoids to be synthesized photolithographically.[8] We employed a technique called digital optical chemistry,[9,10] which involves focusing ultraviolet light onto the synthesis chip by reflection from an array of independently controllable micron-sized polished aluminium micromirrors. This removes the necessity for the tedious and expensive task of creating physical masks for photolithography. In this communication, we report the development of a new chemical strategy that allows the creation of dense arrays of cyclic peptides by digital photolithographic synthesis. Collections of cyclic peptides are excellent sources of high-affinity protein ligands[11,12] and we anticipate that this approach will provide a screening platform of considerable utility.

Libraries of cyclic peptides are usually synthesized by creating a large collection of linear molecules that are then cyclized using appropriate functional groups. However, most cyclization reactions are inefficient, providing a mixture of linear and cyclic molecules, along with oligomers. This type of heterogeneity is undesirable in combinatorial library synthesis. Therefore, we developed a strategy in which a single cyclic framework, cyclo-(Glu(Fmoc)-Dpr(ivDde)-Dpr(NPPOC)-Dpr)-Gly-OH,[13] (Fig. 1A) was synthesized, purified rigorously, then affixed to the surface of the chip, providing a homogeneous cyclic template for subsequent library synthesis. This scaffold includes three chemically orthogonal protecting groups.[14,15] This allows specific deprotection of the NPPOC group using the digital photolithography device only at the features to which one would like to add a particular activated carboxylic acid. Once this is accomplished, the equivalent amino groups on a new set of features could be exposed, then derivatized with a different activated acid. This procedure would be repeated for as many building blocks as desired. Once substitution of that position were complete, all of the base-labile Fmoc protecting groups could be removed with piperidine, then replaced with the photolabile NPPOC protecting group. This would set the stage for another series of spatially segregated photodeprotection steps and activated acid additions. Finally, the ivDde group would be removed by hydrazine, replaced with NPPOC and the same exercise repeated a third time (Fig. 1B).

To test the component steps of this scheme, we first coupled Fmoc-glycine-OH to an amine-coated glass slide, then treated the slide with 10% piperidine in DMF to expose the amino groups. Part of the surface was then coated with NPPOC by treatment with NPPOC-succinimide, while the other part of the glass slide was excluded from such treatment. Next, specific rectangular regions on the surface covered with NPPOC were irradiated with UV light (365 nm, 23 mW cm^{-2}) for different periods of time (Fig. 2A). The entire slide was then reacted with 5(6)-carboxyltetramethylrhodamine, a fluorescent dye able to couple with the primary amine. The ratio of fluorescent intensities between the brightest rectangular region on the surface coated with NPPOC and that excluded from NPPOC-succinimide treatment was used to calculate the combined efficiency of Fmoc conversion to NPPOC, photodeprotection and activated carboxylic acid

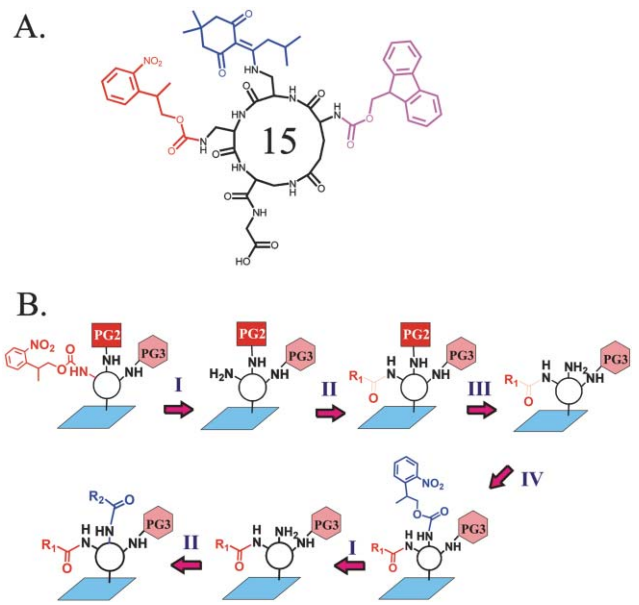

Fig. 1 A. Structure of 15-member cyclic peptide scaffold. The three chemically orthogonal groups are labelled with different colours. B. Synthetic cycle. I. NPPOC group is selectively removed by UV irradiation to expose amine at the desired positions. II. Activated carboxylic acid is coupled to the newly exposed amine. III. One chemically orthogonal protective group is removed to expose free amine. IV. Free amine is protected with the NPPOC group again.

† Electronic Supplementary Information (ESI) available: experimental protocols and an enlarged version of Fig. 3. See http://www.rsc.org/suppdata/cc/b4/b415578e/

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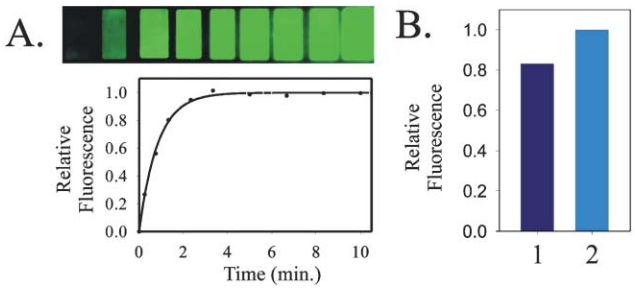

Fig. 2 A. Time-dependent deprotection of NPPOC group on a glass slide. In the upper image, each rectangular area, comprised of NPPOC-protected amines, was irradiated for different times and then labelled with Cy3- phosphoramidite. The bottom is the correlation between the fluorescent intensities of these areas and the time of UV irradiation (365 nm, 23 mW cm^{-2}). B. Efficiency of overall conversion from Fmoc to NPPOC, deprotection, and activated carboxylic acid coupling on a glass slide. Bar 1 is the fluorescent intensity of the area in which the Fmoc was converted to NPPOC and bar 2 is the fluorescent intensity of the area covered with the Fmoc group. The ratio of fluorescent intensities between them was used to calculate the efficiency of the overall conversion.

coupling. These data showed that NPPOC removal was complete in about three minutes and that the overall conversion for all of the chemical and photochemical steps was about 85% (Fig. 2B). Similar optimization experiments were conducted for ivDde with similar results (not shown). We conclude that the protecting group shuffling and photodeprotection steps work well enough to validate pursuing this strategy.

To bring all of the chemistry together, we coupled{cyclo-(Glu(Fmoc)-Dpr(ivDde)-Dpr(NPPOC)-Dpr)-Gly-OH} onto an amine-functionalized glass slide. A "virtual" mask (Mask A, Fig. 3A) with a checkerboard pattern of 4096 features was created to direct UV light to remove NPPOC group on desired features and these newly exposed amines were coupled to activated biotin. The remaining NPPOC groups on the glass slide were then deprotected and the free amines were capped with acetic anhydride. Next, all of the Fmoc groups on the peptide backbone were converted to NPPOC by treating the entire slide with piperidine and NPPOC-succinimide sequentially. The removal of NPPOC and addition of biotin was repeated as above with another "virtual" mask (Mask B) almost identical to Mask A, but containing 8192 features. Again the free amines were coupled to activated biotin. The rest were then photodeprotected and acetylated. Finally, ivDde was exchanged for NPPOC and the array was subjected to another photodeprotection and coupling cycle with a mask of 12 288 features (Mask C). The biotin molecules introduced in these steps were visualized by incubating with a streptavidin-Alexa Fluor 647 conjugate.

If all went as planned, this procedure should have produced a 12 288-feature array, comprised of three groups of 2048 spots containing one, two, or three biotin moieties, respectively, displayed on the cyclic peptide backbone, and the remaining 6144 spots containing no biotin. As shown in Fig. 3B, the fluorescent intensities of these spots correspond qualitatively to the number of biotins on the surface, indicating the precise delivery of monomers to the desired positions.

In conclusion, we have developed a new method to produce a cyclic peptide library that overcomes many problems encountered

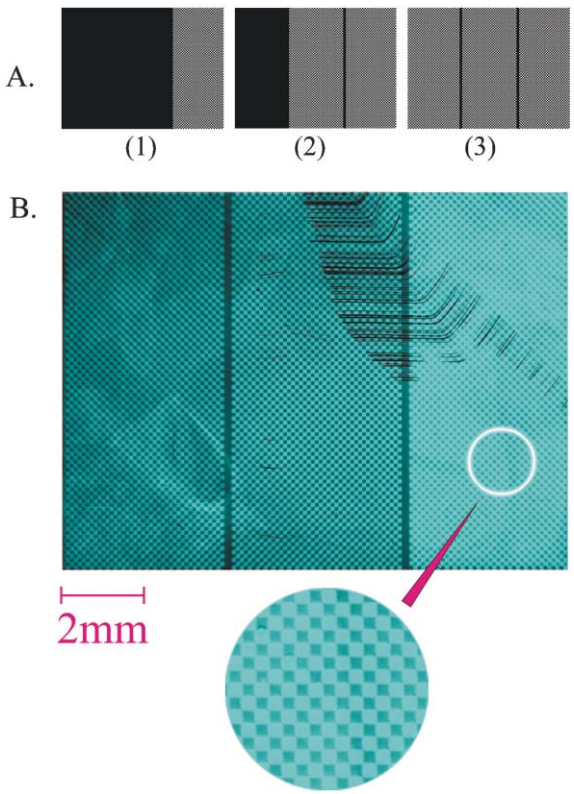

Fig. 3 A. Images of "virtual" masks used. (1) Mask A, (2) Mask B, (3) Mask C. B. Fluorescent image of the model microarray. The size of the microarray is indicated with a scale bar. On the left region, only amine protected by ivDde were coupled with biotin. On middle region, amines protected by both ivDde and Fmoc on cyclic core were coupled with biotin. On the right region, amines protected by ivDde, Fmoc, and NPPOC were coupled with biotin. Part of the microarray is zoomed in to show its fine features. An enlarged, high-resolution image is included in the ESI.†

by current approaches.[16] Since the entire library is built on a cyclic peptide scaffold, the often low-efficiency, sequence-dependent cyclization of thousands of different compounds is avoided. The protecting group swapping strategy allows us to employ a single photolabile protecting group, rather than making large numbers of protected, photolabile monomer units, a major practical impediment until now to the adoption of photolithography as a practical method to synthesize peptide arrays of linear or cyclic molecules. Finally, since the molecules are synthesized in a spatially addressable fashion, there is no need for encoding this array. Current work is focused on employing several different carboxylic side chains to construct complex libraries in this fashion and to screen them for ligands for a variety of proteins.

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Notes and references

1 T. Kodadek, *Chem. Biol.*, 2001, **8**, 105–115.
2 G. MacBeath, A. N. Koehler and S. L. Schreiber, *J. Am. Chem. Soc.*, 1999, **121**, 7967–7968.
3 R. Frank, *J. Immunol. Methods*, 2002, **267**, 13–26.
4 S. P. Fodor, J. L. Read, M. C. Pirrung, L. Stryer, A. T. Lu and D. Solas, *Science*, 1991, **251**, 767–773.
5 R. J. Lipshutz, S. P. Fodor, T. R. Gingeras and D. J. Lockhart, *Nat. Genet.*, 1999, **21**, 20–24.
6 J. P. Pellois, X. Zhou, O. Srivannavit, T. Zhou, E. Gulari and X. Gao, *Nat. Biotechnol.*, 2002, **20**, 922–926.
7 G. H. McGall, A. D. Barone, M. Diggelmann, S. P. Fodor, E. Gentalen and N. Ngo, *J. Am. Chem. Soc.*, 1997, **119**, 5081–5090.
8 S. Li, D. J. Bowerman, N. Marthandan, H. R. Garrer and T. Kodadek, *J. Am. Chem. Soc.*, 2004, **126**, 4088–4089.
9 S. Singh-Gasson, R. D. Green, Y. Yue, C. Nelson, F. Blattner, M. R. Sussman and F. Cerrina, *Nat. Biotechnol.*, 1999, **17**, 974–978.
10 K. J. Luebke, R. P. Balog, D. Mittelman and H. R. Garner, in *Advanced Microfabricated Sensors*, American Chemical Society, Washington, DC, 2002.
11 J. Rizo and L. M. Gierasch, *Annu. Rev. Biochem.*, 1992, **61**, 387–418.
12 R. M. Kohli, C. T. Walsh and M. D. Burkart, *Nature*, 2002, **418**, 658–661.
13 NPPOC: 2-(2-nitrophenyl)propoxycarbonyl. Fmoc: 9-fluorenylmethoxycarbonyl. ivDde: 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl. Dpr: diaminopropionic acid.
14 S. Buhler, I. Lagoja, H. Giegrich, K. P. Stengele and W. Pfleiderer, *Helv. Chim. Acta*, 2004, **87**, 620–659.
15 S. R. Chhabra, B. Hothi, D. J. Evans, P. D. White, B. W. Bycroft and W. C. Chan, *Tetrahedron Lett.*, 1998, **39**, 1603–1606.l.
16 While this work was in progress, we became aware of another effort to construct arrays of cyclic molecules photolithographically. See: A. Shaginian, M. Patel, M.-H. Li, S. T. Flickinger, C. Kim, F. Cerrina and P. J. Belshaw, *J. Am. Chem. Soc.*, 2004, **126**, 16704–16705.