Modulation of Protein-Ligand Interactions by Photo-cleavage of a Cyclic Peptide using Phosphatidylinositol 3-Kinase SH3 Domain as Model System

Short title
Photocleavable cyclic peptide for modifying protein–peptide interaction

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Short Abstract
We synthesized a photocleavable cyclic peptide (cyclic-1) by chemical modification of the SH3 domain interacting peptide with a photolabile linker. The conformation of cyclic-1 was different from the original linear peptide but could be converted to a flexible linear-like structure by UV-irradiation. The binding constant of cyclic-1 with the SH3 domain increased after irradiation. These data suggest that a photocleavable peptide could be used to control the interaction with a protein by modifying the peptide structure.

Abstract
To photomodulate the interaction of the phosphatidylinositol 3-kinase SH3 domain with a peptide ligand, a cyclic peptide (cyclic-1) with a photolabile side chain-to-side chain linker was synthesized. The conformation of cyclic-1 differs from that of the parent linear peptide, but becomes identical by UV-irradiation. Accordingly, the binding affinity of cyclic-1 to the SH3 domain increased upon conversion of the cyclic to a linear flexible structure by irradiation (K_d: 3.4 ± 1.7 and 0.9 ± 0.3 mM, respectively). These results confirm the usefulness of a photocleavable peptide for photocontrol of peptide-protein interactions.

Keywords
Protein−peptide interaction; Phosphatidylinositol 3-kinase SH3 domain; RLP1 peptide; Photocleavage; Cyclic peptide; Photomodulation
INTRODUCTION

Photoactive molecules have attracted much interest, since they can be used with high space and time selectivity [1-21]. For example, photocleavable molecules have been introduced into proteins to study the folding mechanism, whereby the native protein structure is produced instantly by irradiation of the modified protein with light [9-11]. A photoisomerizable intramolecular cross-linker has been used to optically control not only the peptide conformation [12-17] but also its complex formation with biomolecules [18-21]. To optically modify the protein–peptide interaction, we chemically modified a protein interacting peptide with a photolabile linker and produced a photocleavable cyclic peptide (Figure 1a). Cyclization of the peptide restricts its conformation and reduces its interaction with the protein. By light irradiation, the cyclic peptide was converted to a linear flexible peptide, which increased the binding affinity to the protein (Figure 1b).

As a model of the protein–peptide interaction, we utilized the Src homology 3 (SH3) domain of phosphatidylinositol 3-kinase (PI3K) and its peptide ligand, RKLPPRPSK (RLP1) [22]. PI3K is a heterodimeric enzyme composed of a noncatalytic 85 kDa (p85) subunit, which has an SH3 domain, and a catalytic 110 kDa (p110) subunit, which is essential for the kinase activity. It is well-known that the SH3 domain is important in signal transduction and cytoskeletal architecture. The SH3 domain interacts with proline-rich sequences to mediate specific protein–protein interactions [23-25], and the association process between the SH3 domain and the proline-rich peptide has been widely studied, in which long-range electrostatic attraction and hydrophobic interaction play key roles [22, 26-29]. We introduced a cysteine to both N- and C-termini of the acetylated RLP1 peptide (Ac-C-RKLPPRPSK-C; linear-1) and converted the linear peptide to a photocleavable cyclic peptide (cyclic-1) by modification with a photolabile linker, 2,5-bis(bromomethyl)nitrobenzene (Figure 1a). The binding affinity of cyclic-1 to the PI3K SH3 domain was weaker compared to that
of *linear-1*. However, irradiation of *cyclic-1* with light of appropriate wavelength cleaved the peptide at the linker position and produced a *linear-1*-like peptide, which showed an increased binding affinity to the SH3 domain.

**MATERIALS AND METHODS**

*Synthesis of 2,5-bis(bromomethyl)nitrobenzene*

Synthesis of 2,5-bis(bromomethyl)nitrobenzene was performed by stirring the mixture of 2,5-bis(hydroxymethyl)nitrobenzene (0.9 g, 5 mmol), triphenylphosphine (2.6 g, 10 mmol) and carbon tetrabromide (3.3 g, 10 mmol) in anhydrous diethyl ether (20 mL) at 25 °C for 12 h under N₂ atmosphere. The resulting solution was evaporated and purified by column chromatography (silica gel, hexane). After evaporation of the solution, the resulting yellow oil was purified by column chromatography (silica gel, ethyl acetate/hexane, 1:5). Fractions containing the purified product were evaporated and the residue recrystallized from ethyl acetate/hexane. The colorless crystals were collected and dried in vacuo. Yield: 470 mg (30 %). ¹H-NMR (400 MHz, CDCl₃): δ 8.08 (d, 1H), 7.64 (dd, 1H), 7.57 (d, 1H), 4.82 (s, 2H), 4.50 (s, 2H); elemental analysis: calcd. (%) for C₈H₇NBr₂O₂: C 31.10, H 2.28, N 4.53; found: C 31.23, H 2.30, N 4.48.

*Synthesis of cyclic-1*

The linear peptide (Ac-CRKLPYRPSKC, *linear-1*) was synthesized by the solid phase method (Shimadzu). *Linear-1* in 10 mM potassium phosphate buffer (pH 7.4) was mixed with an equimolar amount of 2,5-bis(bromomethyl)nitrobenzene in DMF (buffer/DMF = 9/1 (v/v)). The mixed solution was stirred at 50 °C for 40 min under N₂ atmosphere in the dark to avoid disulfide formation and photocleavage. The crude compound was purified by HPLC using a Shim-pack PREP-ODS(H) column (2 cm φ × 25 cm, Shimadzu). The peptide was eluted at 32.9 min with a linear gradient from 100 % solution A/0 % solution B (0 min) to 80 % A/20 % B (30 min) and finally 70 % A/30 % B (60 min) (flow rate, 1 mL/min; solution A, H₂O with 5 % MeCN and 0.1 % TFA; solution B, 100 % MeCN with 0.1 % TFA). The absorbance of the eluted solution was monitored at 230, 280 and 315 nm. Fractions containing the desired
product were lyophilized and the product was stored in a deep freezer until used. Yield: *ca.* 30%.

**Photocleavage of cyclic-1**

Photo-irradiation of *cyclic-1* in the presence and absence of the SH3 domain was performed in aqueous solution at 4 °C for 20 min by 355-nm pulses obtained from the third harmonic of an Nd:YAG laser (7 mJ, 10 Hz).

**Expression and purification of PI3K SH3 domain**

Unlabelled PI3K SH3 domain was expressed and purified by using the expression vector pLM1 according to the reported method [22]. The $^{15}$N-labelled PI3K SH3 domain was expressed and purified as described below.

The purchased recombinant DNA encoding the His-tagged PI3K SH3 domain (GENE ART) was ligated into pet28a vector and verified by DNA sequencing as follows:

agatctcgatccggaattagttgagcggaattggtgagcggataacaatccccctctagaaataatttttttaacttataaatattacatgtggatgctgtctgtgtcttgattttatccgtctctggggtctttaagaaggagcatctgcatctgggcgatattctgaccgtgaacaaaggcagcctggtgtcggtgcttgctggattttccgggcacctatgtggaatatatcgggcggcaaaaaactcgagcaccaccaaccaccaccacctgagatcgggtgcgagctgctgtgataaacaagtccccgctgctgtgctggctgggttttgtttttttt

$^{15}$N-labelled recombinant PI3K SH3 domain was prepared from culturing freshly transformed *Escherichia coli* BL21 (DE3) cells in M9 minimal medium containing $^{15}$NH$_4$Cl as the sole nitrogen source. Cells were incubated at 37 °C to OD$_{600}$ ≈ 0.8, induced with IPTG (0.5 mM) and allowed to grow for a further 20 h at 22 °C.

The harvested cells were resuspended in 20 mM sodium phosphate buffer (pH 7.0; 500 mM NaCl/1 mM PMSF/50 μg mL$^{-1}$ DNase) and disrupted by sonication. After centrifugation for 1 h at 15000 rpm, the crude extract was loaded onto a Ni affinity chromatography equilibrated with 5 mM imidazole buffer (pH 7.6, 5 mM imidazole/500 mM NaCl/20 mM Tris), washed with 60 mM imidazole buffer and eluted with 500 mM imidazole buffer. Finally, gel filtration chromatography (Superose12 column)
equilibrated with 50 mM sodium phosphate buffer (pH 7.0, including 100 mM NaCl) was performed. Protein concentrations were determined from the absorbance at 280 nm ($\varepsilon = 15.93 \text{ mM}^{-1} \text{ cm}^{-1}$).

**Absorption, CD and mass measurements**

UV-vis and CD spectra were measured with a UV-2450 spectrophotometer (Shimadzu) and a J-720WI spectropolarimeter (Jasco) with 1 cm and 2 mm path-length quartz cells, respectively. Sample conditions are described in each figure caption. MALDI-TOF mass spectra were recorded on a AXIMA-CFR (Shimadzu) in the positive ion reflection mode using α-cyano-4-hydroxycinnamic acid as a matrix. ESI-TOF mass spectra were obtained with a JMS-T100LC AccuTOF (JEOL) in the positive ion mode.

**NMR measurements of the $^{15}$N-labelled SH3 domain**

All NMR experiments were recorded at 298 K on a Bruker DMX600 spectrometer equipped with a TXI-Z-GRAD ($^{1}$H, $^{13}$C, $^{15}$N) probe. NMR samples contained 0.12 mM $^{15}$N-labeled SH3 domain in 50 mM sodium phosphate buffer (pH 7.0, 100 mM NaCl/6% D$_2$O for lock). *Cyclic-1* and photo-irradiated *cyclic-1* were dissolved in 50 mM sodium phosphate buffer (pH 7.0, 100 mM NaCl) to a concentration of 5 mM. For the assignment of amide backbone resonances of the $^{15}$N-labelled SH3 domain, 2D [$^{15}$N,$^{1}$H] HSQC, 3D [$^{15}$N,$^{1}$H] NOESY-HSQC and 3D [$^{15}$N,$^{1}$H] TOCSY-HSQC were recorded. The data were processed in AZARA (http://www.bio.cam.ac.uk/azara).

A series of 2D [$^{15}$N,$^{1}$H] HSQC spectra were recorded with the addition of microliter aliquots of the *cyclic-1* or photo-irradiated *cyclic-1* solution to the SH3 domain sample. Before addition of the peptides, reference 2D [$^{15}$N,$^{1}$H] HSQC spectra were recorded with the free $^{15}$N-labelled SH3 domain. Data processing was performed in AZARA. Chemical shift changes of amide resonances for the $^{15}$N-labeled SH3 domain in the presence of the unlabeled peptides were analyzed by overlaying the spectra of the bound form with the free SH3 domain in ANSIG [30]. After NMR titrations, the sample containing the $^{15}$N-labelled SH3 domain and *cyclic-1* was transferred into an eppendorf tube and irradiated with 365 nm UV/VIS lamp for 20 min.
on ice. Low-intensity 365 nm was obtained from the Spectroline E-series lamp (EF-260C). The irradiated sample was immediately transferred back to an NMR tube and 2D [15N,1H] HSQC spectrum was recorded.

Chemical shift perturbations of amide resonances were plotted against the molar ratio of peptides to protein. A single-site binding model that corrects the influence of the dilution effect was used and a two-parameter nonlinear least-squares fit was performed in the program Origin version 7.5 (Microcal Software, Northampton, MA).

\[
\Delta \delta_{\text{binding}} = \frac{1}{2} \Delta \delta_{\text{max}} \left( 1 - \sqrt{1 - 4R} \right) 
\]

(1)

\[
A = 1 + R + \frac{PR + C}{PCK_a}
\]

(2)
in which \( R \) is the [peptide]/[protein] ratio, \( \Delta \delta_{\text{binding}} \) is the chemical shift perturbation at a given [peptide]/[protein] ratio, \( \Delta \delta_{\text{max}} \) is the chemical shift perturbation at 100 % bound SH3 domain, \( P \) is the initial concentration of the 15N-labelled SH3 domain (0.12 mM), \( C \) is the concentration of the stock peptide solution (5 mM) and \( K_a \) is the association constant, respectively.

The average chemical shift perturbation (\( \Delta \delta_{\text{avg}} \)) of amide resonance was calculated from

\[
\Delta \delta_{\text{avg}} = \sqrt{\frac{(\Delta \delta_{\text{N, binding}} / 5)^2 + \Delta \delta_{\text{H, binding}}^2}{2}}
\]

(3)
in which \( \Delta \delta_{\text{N, binding}} \) and \( \Delta \delta_{\text{H, binding}} \) represent the chemical shift perturbations of amide 15N and amide 1H, respectively.

The observed chemical shift changes at a molecular ratio of [peptide]/[protein] = 8.4 (\( \Delta \delta_{\text{N, binding}}, \Delta \delta_{\text{H, binding}} \)) were extrapolated to 100 % bound protein using the \( K_a \) values from the titrations, and the average values (\( \Delta \delta_{\text{avg, max}} \)) were calculated from eq. (3).

RESULTS AND DISCUSSION

*Cyclic-1* was synthesized by reacting *linear-1* with 2,5-bis(bromomethyl)nitrobenzene and purifying the reaction mixture by reversed phase
HPLC. The MALDI-TOF mass spectrum of cyclic-1 showed a mass peak at 1473.79 (1+), as well as the ESI-TOF mass spectrum at 1473.75 (1+), 737.38 (2+) and 491.92 (3+). These mass peaks were consistent with the calculated mass numbers and isotopic patterns ([M + H]^+ = 1473.75, [M + 2H]^2+ = 737.38 and [M + 3H]^3+ = 491.92; Figure S1). Ellman’s test and Edman degradation of cyclic-1 indicated that there was no free thiol group and no modified amino acid except the cysteine. These results confirm the cyclic structure of the purified peptide. In addition, the CD spectrum of cyclic-1 was clearly different from that of linear-1 (Figure 2a). This difference results from a decrease in the amount of type II polyproline helix making the conformation of cyclic-1 less favorable for binding to the SH3 domain compared to linear-1.

Since cyclic-1 exhibited a broad absorption band around 320 nm (Figure S2), the peptide was irradiated with 355-nm pulses obtained from the third harmonic of a Nd:YAG laser to cleave the photoresponsive linker of cyclic-1. Upon UV-irradiation of cyclic-1 in the presence of DTT, the absorption spectrum immediately showed bands at 272 and 375 nm and then exhibited a band at 309 nm after 2.5 h of irradiation. These absorption changes were similar to the photolytic behavior of caged ATP in the presence of DTT, in which the conversion from a 4-formyl-3-nitrosobenzyl to an anthranyl structure by DTT has been reported [31]. The expected mass peaks of the peptide attached to an anthranyl group were also observed in the ESI-TOF mass spectrum of photo-irradiated cyclic-1 (observed mass numbers: 1457.76 (1+), 729.38 (2+) and 486.58 (3+), calculated ones: [M + H]^+ = 1457.75, [M + 2H]^2+ = 729.38 and [M + 3H]^3+ = 486.59, Figure S3a). The CD spectrum of photo-irradiated cyclic-1 compares well to that of linear-1 (Figure 2a). In the absence of DTT, the ESI-TOF mass spectrum of photo-irradiated cyclic-1 gave the expected mass peaks of the peptide attached to a hydrolytic derivative of the 4-formyl-3-nitrosobenzyl group (observed mass numbers: 1491.74 (1+), 746.38 (2+) and 497.93 (3+), calculated ones: [M + H]^+ = 1491.76, [M + 2H]^2+ = 746.38 and [M + 3H]^3+ = 497.92, Figure S3b). All of these data suggest that cyclic-1 was converted to a linear-1-like structure by UV-irradiation both in the presence and absence of DTT.
The CD spectra of the SH3 domain and cyclic-1 were recorded in 100 mM potassium phosphate buffer (pH 7.0) to determine their interactions. The difference CD spectrum of the SH3 domain complexed with and in absence of linear-1 showed relatively strong bands around 221, 279, 285 and 291 nm and a weaker broad band at 250-300 nm (Figure 2b). This spectral behaviour was similar to that reported for the SH3 domain–RLP1 peptide system, and results from a decrease in the helical content (around 221 nm) and the environmental and/or structural changes of the aromatic residues Tyr12, Tyr14, Tyr73 and Trp55 of the SH3 domain (around 279, 285, 291 and 250-300 nm) induced by association with the peptide [26-28]. As clearly seen in Figure 2b, cyclic-1 did not induce these bands in the corresponding difference CD spectrum, whereas photo-irradiated cyclic-1 did this in a similar manner as linear-1. These observations imply that cyclic-1 binds stronger with the SH3 domain upon photo-irradiation.

Simulation studies suggest that the interaction between the SH3 domain and a proline-rich motif is initiated by long-range electrostatic attraction between negatively charged residues of the protein and the positively charged arginine side chains of the peptide, followed by hydrophobic interactions between ligand and protein together with an interfacial dewetting [29]. When the CD spectra were measured under low ionic strength conditions (10 mM potassium phosphate buffer, Figure S4), photo-irradiated cyclic-1 showed a similar difference spectrum to that of linear-1, indicating strong binding with the SH3 domain. However, under low ionic strength even cyclic-1 gave similar bands (221 (weak), 279, 285 and 291 nm) except for the broad band (250-300 nm). These results allow for three important conclusions: (i) Intermolecular electrostatic interaction plays an important role in binding of both cyclic and linear peptides to the SH3 domain. (ii) Although cyclic-1 and the linear peptide bind at the same site on the SH3 domain, the interactions with the aromatic residues are not entirely the same, since the broad CD band (250-300 nm) is not detected in the cyclic-1 difference spectrum. (iii) The binding constants with the SH3 domain are different between the cyclic and linear peptides (vide infra).
To evaluate the binding constants and the binding sites between the SH3 domain and the peptides, cyclic-1 and photo-irradiated cyclic-1 were titrated into the \textsuperscript{15}N-labeled SH3 domain, and a series of 2D [\textsuperscript{15}N, \textsuperscript{1}H] HSQC spectra were recorded under 100 mM ionic strength. In both cases, significant chemical shift perturbations of the amide resonances of the SH3 domain were observed (Figure 3). HSQC spectrum of photo-irradiated cyclic-1 with the SH3 domain was essentially the same with that obtained by UV-irradiation of the mixture of cyclic-1 and the SH3 domain (Figure 3). These results indicate that addition of the SH3 domain to cyclic-1 during light irradiation does not alter the binding effect of the photo-irradiated peptide. The chemical shift perturbation values for several amide nitrogen of the SH3 domain were plotted against the peptide to SH3 domain molar ratio, and fitted globally to a 1:1 binding model (Figure S5). The estimated dissociation constants ($K_d$) were 3.4 (± 1.7) and 0.9 (± 0.3) mM for cyclic-1 and photo-irradiated cyclic-1, respectively. Therefore, UV-irradiation of cyclic-1 results in a 4-fold stronger binding of the peptide to the SH3 domain. From these dissociation constants, 23 % and 46 % of cyclic-1 and photo-irradiated cyclic-1, respectively, were estimated to bind to the SH3 domain at the peptide to SH3 domain molar ratio of 8.4. The observed amide chemical shift changes at the molar ratio of 8.4 were extrapolated to reflect the estimated changes when 100 % protein is complexed (Figures S6 and S7), and their average values ($\Delta \delta_{\text{avg,max}}$) were color-coded according to the size of their shifts (Figure 4). From these binding maps, the majority of the affected amides laid on the reported peptide binding site of the SH3 domain for both cyclic-1 and photo-irradiated cyclic-1 [22]. Although the yellow color in the binding map of photo-irradiated cyclic-1 seemed to be expanded, the $\Delta \delta_{\text{avg,max}}$ values were similar to those of cyclic-1. In the case of photo-irradiated cyclic-1, the R18 residue was affected significantly by the binding of the peptide, but its signal was not observed at the ratio of peptide/[protein] = 8.4 due to exchange broadening. Accordingly, the peptides interact with similar sites of the SH3 domain, although the precise binding mode is slightly different as evidenced by the differences in the CD spectra.
Since the charges of the two peptides, *cyclic-1* and photo-irradiated *cyclic-1*, are presumably the same at neutral pH, the difference in the dissociation constants should be attributed to the change in the hydrophobic rather than electrostatic interactions. Conformational restriction may also reduce the structural adjustment ability of the peptide for complexation with the SH3 domain, although the conformation stabilized by cyclization may increase the binding ability [32-34]. The photo-irradiated *cyclic-1*, which was converted to the linear flexible structure by light irradiation, should be able to adopt a conformation appropriate for protein binding. However, the calculated dissociation constant of photo-irradiated *cyclic-1* (0.9 mM) were much higher than that of the RLP1 peptide (9.1 μM) [22]. The structural repulsion between the polypeptide chain of the protein and the two cysteine residues and/or the attached benzyl derivatives of the peptide may have caused a weaker binding. This study shows the possibility to modulate the interaction of a photocleavable peptide with a protein by modifying the peptide structure.

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**REFERENCES**


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Figure captions

**Figure 1** Schematic views of (a) production of a photocleavable cyclic peptide and (b) photocontrol of protein–peptide interaction.

**Figure 2** (a) CD spectra of the *linear-1* (black line), *cyclic-1* (blue line) and photo-irradiated *cyclic-1* (red line) Peptide. Sample conditions were 40 μM in an aqueous solution containing 1 mM DTT. (b) Difference CD spectra of the PI3K SH3 domain. The spectra were calculated between with and without *linear-1* (with – without, black line), between with and without *cyclic-1* (with – without, blue line) and between before and after irradiation in the presence of *cyclic-1* (after – before, gray line). The sum of blue and gray lines is shown as a red line, which corresponds to the difference spectrum between with and without photo-irradiated *cyclic-1*. Sample conditions were 10 μM (215-250 nm) and 100 μM SH3 domain (250-315 nm) with and without 4 equivalents of peptide in 100 mM potassium phosphate buffer (pH 7.0) containing 1 mM DTT. Pulse irradiation (7 mJ, 10 Hz) was performed at 4 °C for 20 min for both a and b.

**Figure 3** Region of overlaid 2D [15N, 1H] HSQC spectra: Titration of (a) photo-irradiated *cyclic-1* and (b) *cyclic-1* with the SH3 domain. Free 15N-labelled SH3 domain is represented in black, and peak shifts upon titration of the SH3 domain with increasing amounts of each peptide (red, yellow, green and finally blue). Peak shifts upon irradiation of the solution of the SH3 domain titrated with *cyclic-1* are shown in purple in b.

**Figure 4** Perturbed residue maps of the SH3 domain upon interaction with (a) *cyclic-1* and (b) photo-irradiated *cyclic-1*. The averaged chemical shift changes (Δδ_{avg,max}) are calculated from the values extrapolated to 100 % bound proteins and are color-coded according to the size of shift: Δδ_{avg,max} ≥ 0.35 ppm represented by red, 0.35 > Δδ_{avg,max} ≥ 0.20 ppm by orange, 0.20 > Δδ_{avg,max} ≥ 0.10 ppm by yellow and 0.10 ppm > Δδ_{avg,max} by blue. Gray-colored residues were not assignable.
(a) 
linear-1
(AcC-RKLPPRPSK-C) 
+ 
\[\text{Br-BrNO}_2\] 
\[\text{S-S}\] 
\[\rightarrow\] 
cyclic-1
(b)
Supporting information

“Optical control of protein–peptide interaction using a photocleavable cyclic peptide: Recognition by Phosphatidylinositol 3-kinase SH3 domain”

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Figure S2   Absorption spectra of the peptides  p. 2
Figure S3   ESI-TOF mass spectra of photo-irradiated cyclic-1  p. 3
Figure S4   Difference CD spectra of the PI3K SH3 domain with the peptides  p. 4
Figure S5   Chemical shift perturbations of the SH3 domain upon titration with the peptides  p. 4
Figure S6   Average chemical shift perturbations observed at the ratio of [peptide]/[protein] = 8.4  p. 5
Figure S7   Average chemical shift perturbations extrapolated to 100 % bound protein  p. 5
Figure S1 (a) MALDI-TOF and (b) ESI-TOF mass spectra of cyclic-1. Black lines represent the calculated isotopic patterns of cyclic-1 ([M + H⁺]⁺ = 1473.75, [M + 2H⁺]²⁺ = 737.38 and [M + 3H⁺]³⁺ = 491.92). The mass peak at 1457.63 (1+) in the MALDI-TOF mass spectrum can be attributed to the photoproduct of cyclic-1 by the irradiation with the N₂ laser (337 nm), because its peak intensity increased with the increase of the laser power.

Figure S2 Absorption spectra of the peptides: linear-1 (dotted line), cyclic-1 (black line) and photo-irradiated cyclic-1 (immediately after irradiation, red line; after 2.5 h, blue line). Sample conditions were 20 μM in 10 mM potassium phosphate buffer (pH 7.0) containing 1 mM DTT.
Figure S3 ESI-TOF mass spectra of photo-irradiated cyclic-1 in (a) the presence and (b) absence of DTT. The black lines represent the calculated isotopic patterns of the peptide attached to an anthranil group (a; $[\text{M} + \text{H}^+]^+ = 1457.75$, $[\text{M} + 2\text{H}^+]^{2+} = 729.38$ and $[\text{M} + 3\text{H}^+]^{3+} = 486.59$) and the peptide attached to a hydrolytic derivative of the 4-formyl-3-nitrosobenzyl group (b; $[\text{M} + \text{H}^+]^+ = 1491.76$, $[\text{M} + 2\text{H}^+]^{2+} = 746.38$ and $[\text{M} + 3\text{H}^+]^{3+} = 497.92$).
Figure S4  Difference CD spectra of the PI3K SH3 domain. The spectra were calculated between with and without linear-1 (with – without, black line), between with and without cyclic-1 (with – without, blue line) and between before and after irradiation in the presence of cyclic-1 (after – before, gray line). The sum of the blue and gray lines is shown as a red line, which corresponds to the difference spectrum between with and without photo-irradiated cyclic-1. Sample conditions were the same as those listed in Fig. 2b, except for the buffer concentration (10 mM).

Figure S5  Chemical shift perturbations for amide nitrogen of the SH3 domain upon titration: (a) Photo-irradiated cyclic-1 and (b) cyclic-1. Each symbol (e.g. square, circular, triangular) represents the observed SH3 residue for a given residue number. A 1:1 binding model was used to fit the non-linear curves.
Figure S6 Comparison of the average chemical shift perturbations in each residue of the SH3 domain observed at a molecular ratio of [peptide]/[protein] = 8.4 for photo-irradiated cyclic-1 (filled bar) and cyclic-1 (open bar).

Figure S7 Average chemical shift perturbations extrapolated to 100 % bound protein for the SH3 domain in complex with photo-irradiated cyclic-1 (filled bar) and cyclic-1 (open bar).