Pure Appl. Chem., Vol. 81, No. 2, pp. 273–284, 2009.
doi:10.1351/PAC-CON-08-08-07

Chemical control of biomolecular interaction modules*

Frank Altenbrunn[1], Tom N. Grossmann[1], Christian Haase[1], Franziska Mende[1], Lars Röglin[2], Stefanie Thurley[1], and Oliver Seitz[1,‡]

[1]Institute for Chemistry, Humboldt University, Brook-Taylor-Str. 2, D-12489 Berlin, Germany; [2]Laboratory of Chemical Biology, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands

Abstract: The mutual recognition of biomacromolecules often is mediated by dedicated interaction modules. We take two main approaches in order to recognize and control nucleic acid–nucleic acid, protein–protein, and protein–nucleic acid interactions. In one, the rules that govern the formation of nucleic acid structures are used to design molecules that respond to the presence of nucleic acid or protein targets by showing changes of conformation or reactivity. For example, hybrid molecules can transduce changes of nucleic acid structure to changes of peptide structure, and vice versa. The other approach takes advantage of protein domains that once may form the basis of sensor materials and control elements. However, the current chemical synthesis methods have still not reached the level of maturity required to provide routine access to folded protein domains. In this article, we also describe recent progress that may facilitate the chemical synthesis of protein interaction domains.

Keywords: molecular diagnostics; fluorescent probes; protein–protein interaction; peptide synthesis; switching.

INTRODUCTION

A multitude of mutual recognition events orchestrates the complex networks of interactions between biomacromolecules. These recognition events often are mediated by dedicated interaction modules, which govern nucleic acid–nucleic acid, protein–protein, and protein–nucleic acid binding. One of the chief aims of current research in medicinal chemistry, chemical biology, and synthetic biology is to deliberately interfere with select recognition events. Small-molecule inhibitors of protein–ligand interactions have been used with great success. However, this approach has limitations when the target involves large protein and/or nucleic acid interfaces. A different approach is described in this article. Naturally occurring recognition motifs are considered as role models that are equipped with new functions and fashioned into tools to enable both the detection and a smart control of biomolecular interactions. For example, nucleic acid-driven recognition processes can be used to control chemical reactivity. The ability of nonstructured DNA or RNA to trigger or even catalyze chemical reactions such as ligation reactions or transfer reactions has been demonstrated [1–4]. The reaction products are only formed in the presence of the "correct" DNA or RNA molecule, thereby providing a means to detect

*Paper based on a presentation at the 17th International Conference on Organic Synthesis (ICOS 17), 22–27 June 2008, Daejeon, Korea. Other presentations are published in this issue, pp. 169–298.

‡Corresponding author: Tel.: 49 30 20937446; Fax: 49 30 2093-7266; E-mail: oliver.seitz@chemie.hu-berlin.de

nucleic acid sequences with high sensitivity and unparalleled specificity and sensitivity. Nucleic acid-driven recognition processes can also be used to control the conformation of chimeric molecules. For example, intramolecular hybridization can force a nucleic acid-peptide conjugate to adopt a hairpin structure, which is opened upon binding of a protein to the peptide. The accompanying reorganization of the conformation can result in the separation of two appended fluorophores and can, thus, be detected by changes of fluorescence [5]. Furthermore, nucleic acid hybridization can be employed to control the inhibitory activity of a nucleic acid-peptide conjugate [6]. This approach is interesting as it once may enable a rewiring of cell expression programs. According to this vista, cell endogenous RNA would activate a nucleic acid-peptide conjugate for inhibition of a protein–protein interaction involved in signal transduction. In a paradigm study, peptides that bind to the proteinkinase Src were put under the control of nucleic acid hybridization.

The binding of proteins to other proteins or peptides is governed by dedicated interaction domains. It is one aim to assemble these protein domains on the surface of arrays and to use these arrays for massively parallel protein-based interaction studies. The current methods for the construction of protein arrays rely on the deposition of protein modules from recombinant sources. Chemical synthesis has still not reached the level of maturity that allows the on-chip synthesis of folded protein domains. Fragment ligation techniques may provide a solution to this problem, in particular the most powerful ligation method to date, the native chemical ligation (NCL) [7], has been demonstrated to allow the construction of domain arrays [8]. However, NCL has limitations which constrict the applicability to high-throughput formats. In this article, we describe our recent achievements that may facilitate the chemical synthesis of protein interaction domains [9,10].

Peptide nucleic acids (PNAs) as recognition modules in DNA- and RNA-controlled reactions

DNA- and RNA-controlled reactions are emerging as a general approach to control the reactivity of molecules by modulating their effective molarity [11]. Nonstructured nucleic acid templates align reactive groups of these molecules to allow selective and fast reactions at reactant concentrations that are much lower than those required for conventional synthesis. Several reviews have described applications of DNA- and RNA-controlled synthesis in nucleic acid detection [12,13]. DNA-controlled ligation reactions [1,2,14–24] are plagued by the increased affinity of the ligation product to the target, which results in product inhibition [1,2]. Alternative approaches that allow the formation of products with unchanged affinity involved ester hydrolysis [25–30] and Staudinger reactions [31,32]. In order to be useful for amplified detection, the nucleic acid-directed reactions should proceed rapidly and with low background to deliver an easy to monitor detection signal that is not affected by competing target-independent reaction pathways. The previous detection chemistries have met these criteria only with limited success.

Recently, we presented a general concept for the detection of nucleic acids: the "target-catalyzed transfer of a reporter group" (Fig. 1a) that proved high catalytic activity and holds the potential for various read-out strategies [3,4]. In this approach, two oligonucleotide probes, a donating (**1**) and an accepting (**2**) one are designed to hybridize adjacently to the complementary **target**. The alignment of thioester **1** and the N-terminal *iso*-cysteine **2** in the ternary complex **target·1·2** facilitates a thiol exchange reaction. By analogy to NCL [7], the formed thioester intermediate **4** spontaneously reacts via an irreversible S → N-acyl migration yielding product **5**. Reactant and product probes have similar affinity to the **target**. Thus, strand exchange furnishes ternary complex **target·1·2** in a dynamic equilibrium. In a paradigm study we showed the DNA-catalyzed transfer of a fluorescence quencher (Fig. 1b) [3]. The quencher (Dabcyl) was transferred from a carboxyfluorescein(FAM)- to a carboxytetramethylrhodamin(TMR)-modified PNA probe. The relocation switched on emission of FAM while switching off emission of TMR. This set-up allowed the detection of product formation in

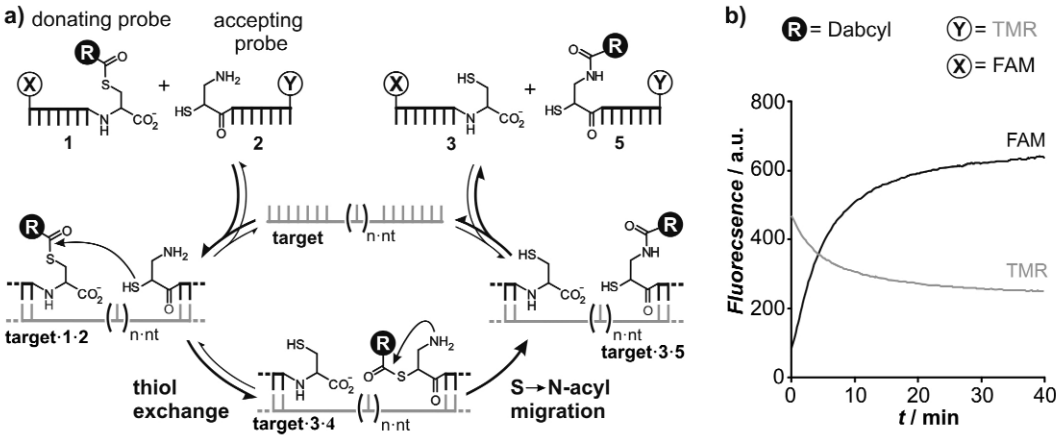

Fig. 1 (a) Catalytic cycle of the target-catalyzed transfer of a reporter group (**R**) from donating **1** to accepting **2** PNA probe. (b) FAM (λ_{ex} = 465 nm, λ_{em} = 525 nm) and TMR (λ_{ex} = 558 nm, λ_{em} = 593 nm) fluorescence of the DNA-directed transfer reaction in the presence of 1 equiv complementary DNA.

real time. After 24 h, we observed in the presence of 0.01 mol % of DNA-catalyst (**target**) a turnover number (TON) of 402, surpassing TON reported for alternative sequence specific methods [1,2].

To increase the sensitivity of the reaction, we used the biotin transfer to preamplify the signal for an enzyme-linked amplification derived from the common enzyme-linked immunosorbent assay (ELISA). We selected HIV-I RNA as **target**. The detection system (Fig. 2a) uses donating PNA probe **6** which contains the biotinylated reporter group and accepting PNA probe **7** bearing a C-terminal His-tag (**His$_6$**). In the presence of **RNA-target** the biotinylated reporter group is transferred (preamplification) yielding products **8** and **9**. Transfer product **9** bears both, a biotin label (**BT**) and a His-tag (**His$_6$**). His-tags are capable of forming strong interactions with nickel ions allowing the immobilization of probe **11** on the surface of Ni-coated well plates. After stringent washing, the wells were incubated with a horseradish peroxidase-streptavidin conjugate (HRP-SA) and washed again. Only in wells with transfer product **9** the HRP-SA conjugate could be immobilized via streptavidin/biotin interaction and form complex **10**. HRP catalyzes the oxidation of colorless tetramethylbenzidin (TMB) to a colored chinoid compound (main amplification). Figure 2b shows background subtracted signals obtained after biotin transfer (preamplification) in the presence of 0.1–5 fmol **RNA-target**, allowing the detection of **target** amounts as low as 500 attomol. Using a common plate reader and reagents that are available in any clinical diagnostics laboratory, this double amplification strategy exceeds the sensitivity obtained by previous nucleic acid-catalyzed chemical reactions [1–3,25–32].

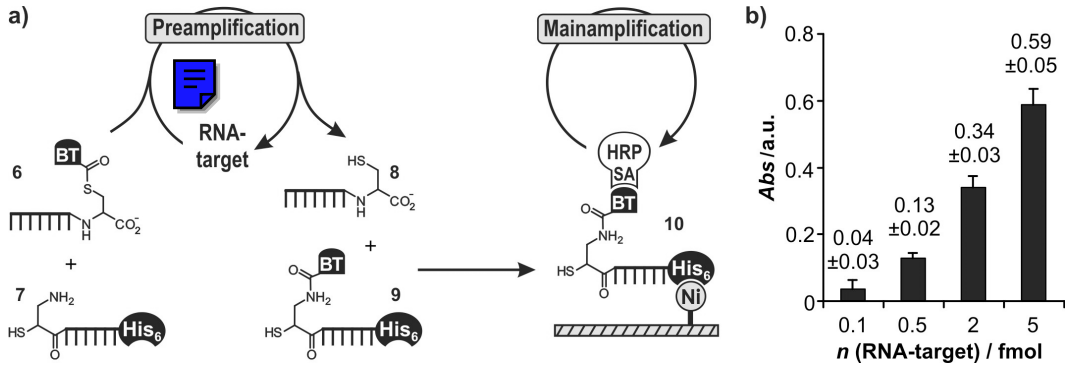

Fig. 2 (a) Double signal amplification using the RNA-catalyzed biotin (**BT**) transfer reaction as preamplification and a modified ELISA as main amplification step. (b) Background subtracted absorbance at 450 nm (triplicate of runs), Conditions: 750 fmol **8**, 500 fmol **9** in 50 µL buffer (preamplification); incubation with 2 µg/mL HRP-SA, 80 µL Ultra-TMB solution followed by addition of 40 µL 1 M H_2SO_4 (main amplification).

Peptide nucleic acids to organize the structure of hairpin peptide beacons (HPBs)

We designed chimeric molecules, so-called HPBs [5], which in analogy to DNA molecular beacons [33] consist of a nucleic acid stem region, two terminally appended chromophores, and a loop region that binds the target. Intramolecular hybridization of two PNA segments provides the stem structure. In contrast to DNA molecular beacons, the loop is spanned by a peptide which is recognized by the target protein. Binding of the target protein to the peptide sequence induces a structural reorganization and results in opening of the closed structure (Fig. 3). The accompanying separation of the terminally appended chromophores leads to increases of fluorescence.

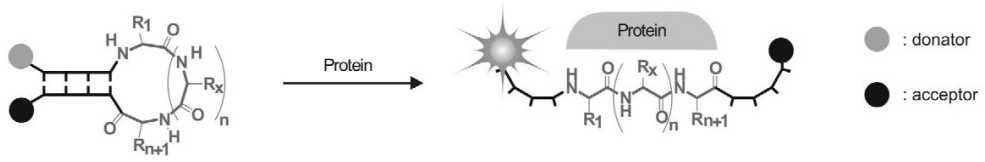

Fig. 3 Hairpin peptide beacons.

The first aim was to develop HPBs that allow detection of the Src-SH2 domain. A known Src-SH2 binder [34] was equipped with self-complementary PNA arms. HPB **11** (Ac-Lys(Pyb)-ttgg-Glu-Pro-pTyr-Glu-Glu-Ile-Pro-ccaa-Lys(Pyb)Gly-NH_2) was prepared by Fmoc-based solid-phase synthesis, and the ε-amino groups of terminal lysine residues were labeled with pyrenebutanoic acid (Pyb). Addition of the target Src-SH2 protein evoked a rapid 11-fold fluorescence increase (Fig. 4a). In contrast, control experiments with the SH2 domain of the adaptor protein Grb2 resulted only in small fluorescence responses. In addition, it was shown that structuring the peptide sequence by using PNA has a positive influence on the selectivity of HPBs compared to the peptide beacon (PB) **13** Ac-Lys(Pyb)-Glu-Pro-pTyr-Glu-Glu-Ile-Pro-Lys(Pyb)-Gly-NH_2. Fluorescence titrations confirmed the increased selectivity of the structured HPBs. For example, the HPB **11**·Src-SH2 complex had a dissociation constant K_d = 1.5 µM, which is more than two-fold lower than the K_d = 3.5 µM measured for the HPB **11**·Grb2-SH2. By contrast, the affinity of the PB **13** for Src-SH2 (K_d = 4.5 µM) was as high as the affinity for Grb2-SH2 (K_d = 4.4 µM).

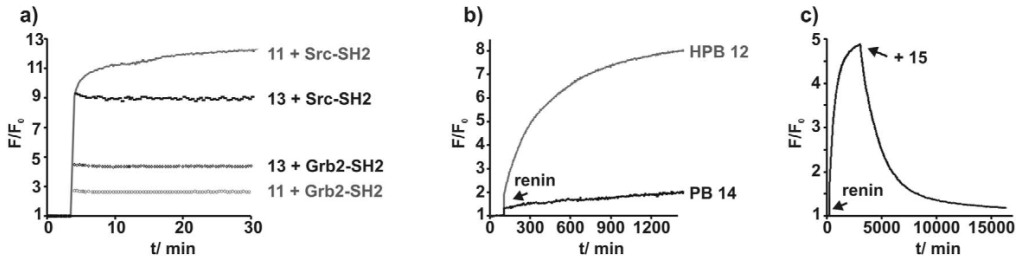

Fig. 4 Time course of (a) relative fluorescence intensity at 380 nm of HPB **11** (gray) or PB **13** (black) after addition (5 min) of Src-SH2 and Grb2-SH2; (b) of relative fluorescence emission at 690 nm (NIR fluorescence) of HPB **12** (100 nM) and PB **14** (100 nM), respectively, in buffer after addition of 120 nM renin (for **12**) and 600 nM renin (for **14**) and (c) of relative fluorescence of HPB **12** (100 nM) after addition of renin. After 25 min, 0.3 mM Ac-Pro-His-Pro-Phe-His-Statine-Val-Ile-Gly-NH2, **15** was added to the protease-HPB complex.

To explore the versatility of the HPB design, we explored the protease renin. It was the aim to develop a probe that, in contrast to most other protease reporters, would not be subject to cleavage. The HPB **12** [Ac-Lys(NIR)-ttgg-Pro-His-Pro-Phe-His-Stat-Val-Ile-ccaa-Lys(Dabcyl)-Gly-NH_2] wherein the amino acid analogon statine (Stat) replaced the natural cleavage site Val-Leu or Leu-Leu of renin substrates, was found to signal the presence of renin by up to 8-fold increases of NIR-667 emission (Fig. 4b). By contrast, only 2-fold fluorescence enhancement was observed for the nonstructured peptide probe **14** [Ac-Lys(NIR)-Pro-His-Pro-Phe-His-Stat-Val-Ile-Lys(Dabcyl)-Gly-NH_2] despite exposure to an increased renin concentration. Addition of a competitor peptide (**15**: Ac-Pro-His-Pro-Phe-His-Stat-Val-Ile-Gly-NH_2) to the HPB **12**-protease complex (Fig. 4c) decreased the fluorescence, which provided ample evidence for the reversibility of fluorescence signaling. Thus, we propose that HPBs may allow the continuous monitoring of protein expression. Experiments that address the generality and specificity of HPB signaling are currently ongoing.

PNA-peptide conjugates to enable switching of protein activity with DNA and RNA hybridization

Switching of the activity of peptides and proteins is a key biological process which modulates the function of proteins in protein–protein interaction networks. The importance of this process in cellular signaling has stimulated research in the design of switchable peptide conjugates [35]. In analogy to cellular signaling, the reception of an external stimulus is envisioned to change the conformational state of the peptide, and thus its biological activity. Many efforts have been made to use light as an external stimulus. In these approaches, photoresponsive groups such as azobenzene moieties are coupled to the backbone or the side chains of amino acids in order to control peptide activity in vitro and in vivo [36–38]. We explore switching processes that are triggered by nucleic acid-based binding interactions. The goal is to develop a strategy that puts a specific protein–protein interaction under the control of nucleic acid hybridization such that a particular mRNA molecule confers the inhibition of a protein–protein interaction involved in signal transduction. Achievements toward this goal may once enable a rewiring of cell expression programs. The envisioned approach requires a transducer, and we reckoned that hybridization of nucleic acid-peptide conjugates with DNA or RNA may be used to alter the constraints that are experienced by the peptide part [6]. The peptide may respond by showing changes of the affinity for a protein target. The peptide of interest is equipped with noncomplementary PNA arm segments which flank the C-and the N-terminus (Fig. 5). The addition of DNA or RNA to the PNA-peptide-PNA chimera leads to the formation of a double-stranded complex that may induce a structural reorganization. For example, contiguous base-pairing that involves both PNA arms may increase the tendency to adopt the loop-like structure in PNA-peptide-PNA·DNA **16** complex. Other DNA or RNA

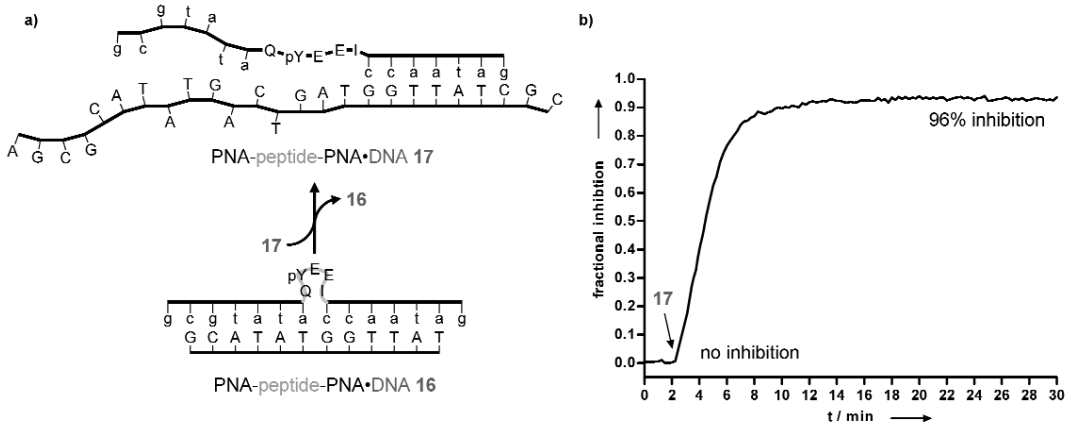

Fig. 5 Hybridization-directed control of peptide activity. The complexes formed upon addition of complementary DNA or RNA to unstructured PNA-peptide hybrid restrict the conformational flexibility of the peptide (QpYEEI). The hybrid is unable to inhibit the binding of Src-SH2 with a reference peptide when complexed by DNA **16**. Addition of DNA **17** induces a strand exchange. The peptide more readily adopts the required extended conformation and is able to inhibit binding of reference peptide FAM-Gly-pTyr-Glu-Glu-Ile-Ala-NH_2 to Src-SH2.

sequences that bind only one PNA arm may increase the tendency to adopt extended conformations, e.g., in PNA-peptide-PNA·DNA **17** duplex.

In a paradigm study, we have shown that nucleic acid hybridization can act as a trigger to reversibly increase or decrease, at will, the affinity of a PNA-phosphopeptide-PNA chimera to the SH2 domain of the Src kinase [6]. This SH2 domain binds phosphopeptides that contain the recognition motif pTyr-Glu-Glu-Ile in an extended conformation. The nonmodified phosphopeptide Ac-Gln-pTyr-Glu-Glu-Ile-NH_2 inhibits the binding of the reference peptide FAM-Gly-pTyr-Glu-Glu-Ile-Ala-NH_2 to Src-SH2 with an IC_{50} = 4.6 ± 0.9 μM. The affinity of single-stranded PNA-peptide chimera for Src-SH2 was reduced by 50 %. We have shown that constraints that favor a bulged peptide structure such as in duplex PNA-peptide-PNA·DNA **16** resulted in further 50 % decreases of binding affinities. Remarkable increases of binding affinities were demonstrated in strand exchange experiments. For example, addition of DNA **17** afforded the more stable duplex PNA-peptide-PNA·DNA **17**. In this duplex, the phosphopeptide more likely adopts the required extended conformation than in the duplex with DNA **16**. Indeed, PNA-peptide-PNA·DNA **17** has a 7-fold higher affinity for Src-SH2 than the nonmodified phosphopeptide Ac-Gln-pTyr-Glu-Glu-Ile-NH_2. The observed differences in binding affinities comprise one order of magnitude. Thus, it proved feasible to switch from nearly no inhibition to almost quantitative inhibition of the interaction of Src-SH2 with the peptide ligand (Fig. 5).

In cells, binding of phosphopeptides to the Src-SH2 domain acid serves to increase the activity of the autoinhibited Src-kinase [39]. We envision that conjugates such as PNA-peptide-PNA·DNA **16** can become activated by cell endogenous RNA (having a sequence like DNA **17**). Expectedly, the PNA-peptide-PNA·DNA **16** was found to have little effect on the phosphorylation activity of autoinhibited Src-kinase. However, the addition of an RNA molecule (having a sequence like DNA **17**) resulted in almost 90 % reactivation, presumably by activating the binding affinity of the PNA-peptide-PNA chimera in a complex similar to PNA-peptide-PNA·DNA **17**.

Extending the applicability of NCL: Peptide fragment ligations at valine

NCL is a powerful method that provides reliable access to proteins and protein domains [7,40]. The NCL technique allows the merger of two unprotected peptide segments and furnishes an amide bond which is formed in the reaction of a peptide thioester and a cysteine peptide. In our pursuit of the chemical synthesis of protein domain arrays, we also take advantage of this very useful reaction. However, cysteine is one of the least frequent amino acids in nature and there are protein domains which do not contain a single cysteine residue. Different strategies have been developed to overcome this paucity. The cysteine-free ligation makes use of N-terminally attached auxiliaries that mimic the cysteine scaffold. Most auxiliary groups include electron-rich aromatic systems to ensure a traceless acidolytic removal [41,42]. However, the increased steric demand at the secondary amine structure reduces the ligation rate, and this may be one reason why all reported examples involved glycine-containing ligation sites. An alternative approach makes use of a ligation-desulfurization sequence at β-thiol amino acids wherein the initial native peptide coupling is followed by the selective removal of the accessory sulfur atom. In this strategy, cysteine can be used as precursors of the abundant amino acids alanine [43] and serine [44]. The synthesis of a β-mercapto-phenylalanine building block has provided access to phenylalanine-containing peptide bonds [45].

We explored the use of penicillamine as a commercially available precursor to valine [10]. Valine occurs in proteins with high frequency. In addition, we envisioned that NCL at valine would grant access to hydrophobic ligation sites which have not been available by the existing methods. In the initial step of the penicillamine-ligation the tertiary mercapto moiety of peptide **18** displaces the thiol component of the peptide thioester **19**. The subsequent acyl shift converts the intermediate thioester **20** to the penicillamine peptide **21**. Final desulfurization provides the valine residue in **22**.

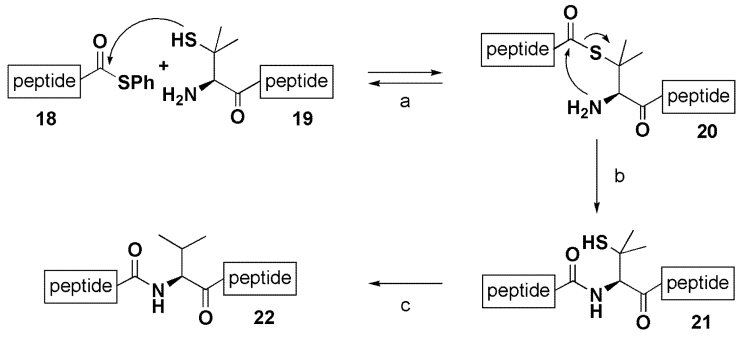

Fig. 6 NCL at valine. The tertiary thiol moiety in **19** reversibly exchanges the thiol component of **18**. (step a) Subsequently, thioester intermediate **20** undergoes a S,N-acyl migration that furnishes the amide bond in **21**. (step b) Removal of the surplus sulfur atom provides the valine side chain in **22**.

Initially, we anticipated that the steric shielding of the methyl groups would hinder access to the tertiary thiol group in **19**. However, we imagined that the increased nucleophilicity of the penicillyl thiol group would provide partial compensation. Indeed, the reaction of the peptide thioester H-Leu-Tyr-Lys-Ala-Gly-$SCH_2CH_2CONH_2$ with penicillyl peptide H-Pen-Arg-Ala-Glu-Tyr-Ser-NH_2 proceeded with remarkable efficiency affording the coupling product H-Leu-Tyr-Lys-Ala-Gly-Pen-Arg-Ala-Glu-Tyr-Ser-NH_2 in 60 % yield after 1 h and 87 % yield after 12 h reaction time when performed at pH 8.0 and with thiophenol as sole thiol additive. We subsequently increased the steric demand at the ligation site and explored ligations which involved histidin and methionine as C-terminal amino acid of the thioester component. We were pleased to see that the desired products were obtained in 70 and 65 % yields, respectively, after 24 h. Even the reaction of the leucine thioester H-Leu-Tyr-Lys-Ala-Leu-SPh proceeded

smoothly and furnished the bulky Leu-Pen peptide bond in 82 % yield after 48 h. Furthermore, the usefulness of ligation at pencillylpeptides was demonstrated in the coupling of two longer, naturally occurring peptide fragments (Ac-Leu-Lys-Lys-Pro-Phe-Asn-Arg-Pro-Gln-Gly-SBn + H-Pen-Gln-Pro-Lys-Thr-Gly-Pro-Phe-Glu-asp-Leu-Lys-NH_2), which proceeded with quantitative conversion.

For the final step of the ligation-desulfurization sequence, we initially applied metal-based methods (such as reduction with Raney nickel) to achieve a reduction of the thiol moeties. However, tedious purification and adsorption of the peptides to the metal surfaces rendered this approach cumbersome. We therefore employed a recently disclosed method in which radical-based reactions [46] are used to convert cysteinyl- to alanyl-peptides. The radical process is initiated by a water-soluble radical starter such as VA-044, which homolytically abstracts the hydrogen of the thiol function. A subsequent reaction of the formed thiyl radical species with tris(2-carboxylethyl)phosphan (TCEP) is expected to afford an alkyl radical intermediate that is trapped via a hydride transfer from the highly potent hydride donor glutathione. This reaction provided reliable access and high yields of the desired valine peptide.

In concluding, we have demonstrated access to hydrophobic, valine-containing ligation sites by using penicillamine in the ligation-desulfurization strategy. The necessary building block is commercially available and offers a new option for peptide ligation to protein chemistry.

Solid-phase synthesis with self-purification: Improved access to peptidethioesters for NCL

The efficiency of chemical peptide synthesis on array surfaces is currently not sufficient to enable access to homogeneous, immobilized protein domains. Fragment ligation methods will probably provide a solution to this problem. The most powerful fragment coupling method to date relies on NCL of peptide thioesters with cysteinylpeptides [7]. However, the available methods for solid-phase synthesis of peptide thioesters often require additional reaction steps in solution and are therefore not adapted to high-throughput formats [47–53]. In addition, purification of the crude peptide thioesters is time-consuming, sometimes even cumbersome owing to side-reactions of the active esters. We designed a method for Fmoc-based synthesis of peptide thioesters that avoids the need for high-performance liquid chromatography (HPLC) purification [9]. Only the full-length peptide thioesters are selectively detached and directly used in NCL (Fig. 7).

Inspired by cyclization-cleavage strategies used for inversion of peptide orientation on solid support [54–57], we developed a reaction sequence that includes an on-resin macrocyclization via the N-terminus, a thiolysis-induced ring opening at sulfonamide "safety-catch" linker and a standard trifluoroacetyl (TFA) cleavage protocol. This reaction sequence is commenced with the attachment of cyclization linker **23** to peptide **24**, which was assembled on a sulfonamide "safety-catch" resin by using the Fmoc/*t*Bu-strategy. **23** carries an allyloxycarbonyl(Aloc)-protected amino function for subsequent macrolactamization. Alkylation of the acyl-sulfonamide in **25** introduced the allyl-protected ester of **27**. Pd(0)-catalyzed removal of the allyl groups allowed the subsequent macrolactamization, which provided macrocycle **28**. The ring was opened upon thiolytic cleavage of the alkylated N-acyl sulfonamide bond. Capped truncation products do not carry the cyclization linker and are excluded from macrolactamization. Hence, only the full-length peptide thioesters **29** remain on the resin, while the truncated sequences **30** are released into solution at this state of synthesis. The final TFA treatment leads to fully deprotected peptide thioesters **31**.

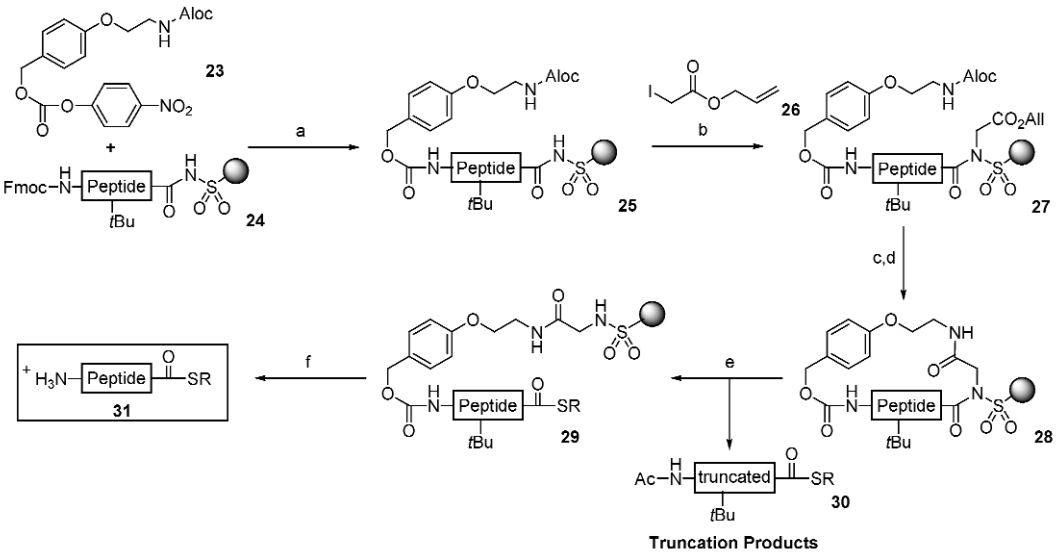

Fig. 7 Fmoc-based solid-phase synthesis of peptide thioesters with self-purification. (a) **23**, 5 % NEt_3/DMF, (b) alkylation: **26**, DIPEA, DMF, (c) deallylation: Pd(0), *N*,*N*'-dimethylbarbituric acid, (d) macrolactamization: PyBOP, (e) thiolysis: RSH, NaSPh, (f) TFA cleavage. Aloc = allyloxycarbonyl, All = allyl, DIPEA = ethyldiisopropylamine, PyBOP = benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate.

The synthesis of several peptide thioesters proved the efficiency of the cyclization-thiolysis approach. The crude products were characterized by HPLC and compared with crude products which were obtained in a "conventional" peptide thioester synthesis on the sulfonamide resin (Fig. 8).

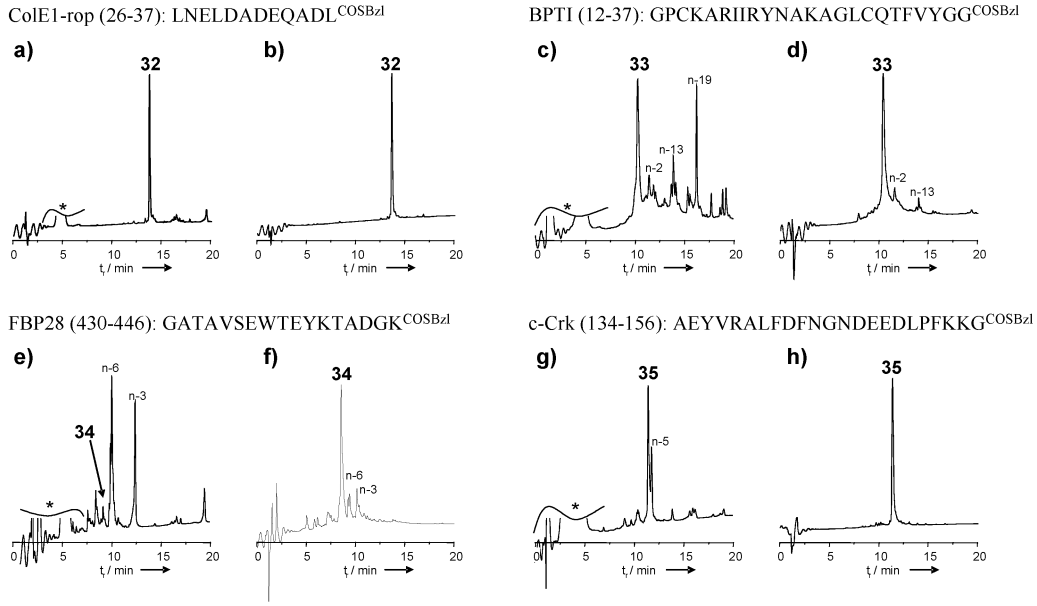

Fig. 8 HPLC traces of crude peptide thioesters **32–35** in (a), (c), (e), and (g) obtained by conventional approach (* NaSPh and DMF) and in (b), (d), (f), and (h) obtained by peptide thioester synthesis with self-purification.

Peptide sequence **32** is derived from the segment 26–37 ColE1 repressor of primer protein. Although only small amounts of truncation products are detected in crude **32** synthesized by the linear approach (Fig. 8a), purification by HPLC was necessary due to contamination with NaSPh used in thiolysis of the acyl sulfonamide bond. **32** was isolated in 8 % yield and 92 % purity. Following the peptide thioester synthesis with self-purification, crude **32** was obtained in 20 % yield and 97 % purity (Fig. 8b). The self-purification method was put to test in the synthesis of the two difficult peptide sequences **33** and **34**. The conventional synthesis provided the BPTI thioester 12–37 **33** in a mixture with many truncation products (Fig. 8c). Purification was cumbersome and caused problems due to intramolecular thioester exchange. **33** was obtained in 6 % yield and only 68 % purity. The self-purification approach furnished crude **33** in 18 % yield and 77 % purity (Fig. 8d). Linear synthesis of the peptide segment 430–446 of WW domain of formin binding protein 28 totally failed. The crude product contained only trace amounts of the full-length peptide thioester **34** (Fig. 8e). By contrast, the cyclization-thiolysis strategy **34** afforded a crude product that contained **34** as major product in 54 % purity (Fig. 8f) but only in 3 % yield. A sequence of the SH3 domain of c-Crk adaptor protein (134–156) was synthesized. Again, peptide thioester synthesis with self-purification (Fig. 8h) showed better results in terms of yield and purity than the conventional approach (Fig. 8g). **35** was accessed in 30 % yield and 98 % purity and was afterwards successfully used in NCL to generate the full-length SH3 domain (134–191).

CONCLUSION

Our aim is to take advantage of naturally occurring biomolecular interaction modules and to use these modules to construct sensor materials and control elements that may be applied even in live cells. The rules that govern the formation of nucleic acid structures are relatively well understood. In addition, chemical access to meaningful nucleic acid-based molecules is straightforward. We demonstrated that nucleic acid-based interactions can be used to control chemical reactivities. In one example, we showed that it is feasible to develop reactions that occur only when catalytic amounts of nonstructured DNA or RNA targets are present. The catalytic efficiency allowed us to increase the sensitivity of a DNA/RNA detection assay. In another example, we used chimeric molecules that contain a nucleic acid part and a peptide part. In these molecules, nucleic acid-based interactions served to constrain the peptide. For example, HPBs were designed as stem-loop probes in which intramolecular hybridization forces the peptide part to adopt a loop structure. The hairpin-shaped molecules were demonstrated to undergo a conformational reorganization upon binding of a target protein to the peptide. This process was monitored by measuring the changes of fluorescence of terminally appended dyes. Intermolecular hybridization provides the opportunity to perform switch processes. We showed that addition of DNA molecules can induce both activation and inactivation of binding of a signal transduction protein, and we envision that such switch processes could be induced by cell-endogenous RNA.

In the pursuit of using protein domains as construction units of sensor materials, we are still in the phase of improving the synthetic access to such modules. The NCL of unprotected peptide segments probably is amongst the most advanced synthesis methods that have provided access to various proteins and protein domains. Yet, there are limitations, which have been addressed in this article. We have demonstrated that the NCL can be performed on more abundant amino acids than cysteine and added valine to the repertoire of accessible ligation sites. We also introduced a new method for the Fmoc-based synthesis of peptidethioesters that proceeds with self-purification, and we proposed the application in the high-throughput preparation of peptide thioesters for direct use in on-array fragment ligations.

REFERENCES

1. H. Abe, E. T. Kool. *J. Am. Chem. Soc.* **126**, 13980 (2004).
2. C. Dose, S. Ficht, O. Seitz. *Angew. Chem., Int. Ed.* **45**, 5369 (2006).
3. T. N. Grossmann, O. Seitz. *J. Am. Chem. Soc.* **128**, 15596 (2006).
4. T. N. Grossmann, L. Röglin, O. Seitz. *Angew. Chem., Int. Ed.* **47**, 7119 (2008).
5. S. Thurley, L. Röglin, O. Seitz. *J. Am. Chem. Soc.* **129**, 12693 (2007).
6. L. Röglin, M. R. Ahmadian, O. Seitz. *Angew. Chem., Int. Ed.* **46**, 2704 (2007).
7. P. E. Dawson, T. W. Muir, I. Clark-Lewis, S. B. H. Kent. *Science* **266**, 776 (1994).
8. F. Toepert, T. Knaute, S. Guffler, J. R. Pires, T. Matzdorf, H. Oschkinat, J. Schneider-Mergener. *Angew. Chem., Int. Ed.* **42**, 1136 (2003).
9. F. Mende, O. Seitz. *Angew. Chem., Int. Ed.* **46**, 4577 (2007).
10. C. Haase, H. Rhode, O. Seitz. *Angew. Chem., Int. Ed.* **47**, 6807 (2008).
11. X. Y. Li, D. R. Liu. *Angew. Chem., Int. Ed.* **43**, 4848 (2004).
12. A. P. Silverman, E. T. Kool. *Chem. Rev.* **106**, 3775 (2006).
13. T. N. Grossmann, A. Strohbach, O. Seitz. *ChemBioChem* **9**, 2185 (2008).
14. A. Mattes, O. Seitz. *Chem. Commun.* 2050 (2001).
15. A. Mattes, O. Seitz. *Angew. Chem., Int. Ed.* **40**, 3178 (2001).
16. S. Ficht, A. Mattes, O. Seitz. *J. Am. Chem. Soc.* **126**, 9970 (2004).
17. S. Ficht, C. Dose, O. Seitz. *ChemBioChem* **6**, 2098 (2005).
18. C. Dose, O. Seitz. *Bioorg. Med. Chem.* **16**, 65 (2008).
19. Y. Z. Xu, E. T. Kool. *Tetrahedron Lett.* **38**, 5595 (1997).
20. Y. Xu, E. T. Kool. *Nucleic Acids Res.* **27**, 875 (1999).
21. Y. Z. Xu, N. B. Karalkar, E. T. Kool. *Nat. Biotechnol.* **19**, 148 (2001).
22. S. Sando, E. T. Kool. *J. Am. Chem. Soc.* **124**, 2096 (2002).
23. S. Sando, E. T. Kool. *J. Am. Chem. Soc.* **124**, 9686 (2002).
24. S. Sando, H. Abe, E. T. Kool. *J. Am. Chem. Soc.* **126**, 1081 (2004).
25. Z. C. Ma, J. S. Taylor. *Proc. Natl. Acad. Sci. USA* **97**, 11159 (2000).
26. Z. C. Ma, J. S. Taylor. *Bioorg. Med. Chem.* **9**, 2501 (2001).
27. J. F. Cai, X. X. Li, J. S. Taylor. *Org. Lett.* **7**, 751 (2005).
28. J. Brunner, A. Mokhir, R. Krämer. *J. Am. Chem. Soc.* **125**, 12410 (2003).
29. F. H. Zelder, J. Brunner, R. Krämer. *Chem. Commun.* 902 (2004).
30. I. Boll, R. Krämer, J. Brunner, A. Mokhir. *J. Am. Chem. Soc.* **127**, 7849 (2005).
31. J. F. Cai, X. X. Li, X. Yue, J. S. Taylor. *J. Am. Chem. Soc.* **126**, 16324 (2004).
32. Z. L. Pianowski, N. Winssinger. *Chem. Commun.* 3820 (2007).
33. S. Tyagi, F. R. Kramer. *Nat. Biotechnol.* **14**, 303 (1996).
34. G. Waksman, S. E. Shoelson, N. Pant, D. Cowburn, J. Kuriyan. *Cell* **72**, 779 (1993).
35. O. Pieroni, A. Fissi, N. Angelini, F. Lenci. *Acc. Chem. Res.* **34**, 9 (2001).
36. I. Willner. *Acc. Chem. Res.* **30**, 347 (1997).
37. L. Guerrero, O. S. Smart, C. J. Weston, D. C. Burns, G. A. Woolley, R. K. Allemann. *Angew. Chem., Int. Ed.* **44**, 7778 (2005).
38. C. Renner, L. Moroder. *ChemBioChem* **7**, 869 (2006).
39. S. J. Parsons, J. T. Parsons. *Oncogene* **23**, 7906 (2004).
40. P. E. Dawson, S. B. H. Kent. *Annu. Rev. Biochem.* **69**, 923 (2000).
41. J. Offer, C. N. C. Boddy, P. E. Dawson. *J. Am. Chem. Soc.* **124**, 4642 (2002).
42. P. Botti, M. R. Carrasco, S. B. H. Kent. *Tetrahedron Lett.* **42**, 1831 (2001).
43. L. Z. Yan, P. E. Dawson. *J. Am. Chem. Soc.* **123**, 526 (2001).
44. R. Okamoto, Y. Kajihara. *Angew. Chem., Int. Ed.* **47**, 5402 (2008).
45. D. Crich, A. Banerjee. *J. Am. Chem. Soc.* **129**, 10064 (2007).
46. Q. Wan, S. J. Danishefsky. *Angew. Chem., Int. Ed.* **46**, 9248 (2007).

47. R. Ingenito, E. Bianchi, D. Fattori, A. Pessi. *J. Am. Chem. Soc.* **121**, 11369 (1999).
48. J. Brask, F. Albericio, K. J. Jensen. *Org. Lett.* **5**, 2951 (2003).
49. S. Futaki, K. Sogawa, J. Maruyama, T. Asahara, M. Niwa, H. Hojo. *Tetrahedron Lett.* **38**, 6237 (1997).
50. R. von Eggelkraut-Gottanka, A. Klose, A. G. Beck-Sickinger, M. Beyermann. *Tetrahedron Lett.* **44**, 3551 (2003).
51. L. Li, P. Wang. *Tetrahedron Lett.* **48**, 29 (2007).
52. J. Alsina, T. S. Yokum, F. Albericio, G. Barany. *J. Org. Chem.* **64**, 8761 (1999).
53. J. B. Blanco-Canosa, P. E. Dawson. *Angew. Chem., Int. Ed.* **47**, 6851 (2008).
54. R. S. Kania, R. N. Zuckermann, C. K. Marlowe. *J. Am. Chem. Soc.* **116**, 8835 (1994).
55. U. Hoffmüller, M. Russwurm, F. Kleinjung, J. Ashurst, H. Oschkinat, R. Volkmer-Engert, D. Koesling, J. Schneider-Mergener. *Angew. Chem., Int. Ed.* **38**, 2000 (1999).
56. M. Davies, M. Bradley. *Angew. Chem., Int. Ed.* **36**, 1097 (1997).
57. M. Davies, M. Bradley. *Tetrahedron* **55**, 4733 (1999).