

Metal-Chelating Amino Acids As Building Blocks For Synthetic Receptors Sensing Metal Ions And Histidine-Tagged Proteins

Silke Hutschenreiter, Lars Neumann, Ulf Rädler, Lutz Schmitt, and Robert Tampé^{*,[a]}

Protein structure and function rely on a still not fully understood interplay of energetic and entropic constraints defined by the permutation of the twenty genetically encoded amino acids. Many attempts have been undertaken to design peptide–peptide interaction pairs and synthetic receptors de novo by using this limited number of building blocks. We describe a rational approach to creating a building block based on a tailored metal-chelating

amino acid. Nε,Nε-bis(carboxymethyl)-L-lysine can be flexibly introduced into peptides by 9-fluorenylmethoxycarbonyl solid-phase chemistry. The corresponding metal-chelating peptides act as metal sensors and synthetic receptors for histidine-tagged proteins. These biochemical tweezers will open new ways to control protein–protein interactions, to design peptide-based interaction pairs, or to generate switchable protein function.

Introduction

Modern techniques in structural biology have increased our knowledge of the structure and function of biomolecules exponentially. However, the design of a protein with defined or modified function(s) or secondary structure elements such as α helices or β sheets is still a challenging task.^[1, 2] Nevertheless, success in designing coiled coils,^[3, 4] helical bundles,^[5, 6] β -turns,^[7, 8] and hairpins^[9, 10] has demonstrated that, in principle, de novo design of proteins or protein–protein interactions is feasible. A general approach for the rational design of peptides with novel functions, protein-mediated recognition processes, or protein–protein interactions is extremely desirable but is still not in sight. In general, recognition processes are determined by a complex superposition of many weak long- and short-range forces (electrostatic, van der Waals, and hydrogen bonds) as well as entropic constraints, which in concert are difficult to predict and to control. Alternatively, ligands can be coordinated precisely in a three-dimensional arrangement by complex formation. For example, $(\text{Ni}^{2+}:\text{nitrilotriacetic acid})_n$ derivatives of cyanine fluorochromes can function as fluorescence resonance energy transfer (FRET) acceptors for fluorescein-labeled histidine-tagged DNA–protein complexes and proteins in a distance-dependent manner.^[11] Herein we describe a synthetic amino acid that can be incorporated into any polypeptide by solid-phase peptide synthesis. Potential applications of these metal-chelating units as metal sensors and building blocks for synthetic receptors that interact specifically with histidine-tagged peptides and histidine fusion proteins are discussed.

Results and Discussion

Building blocks for the coordination of metal ions and their incorporation into peptides or proteins have already been

described.^[12–18] However, in most cases several amino acids are necessary to form stable metal complexes. In our approach, one metal-chelating side chain is sufficient and can bind in a subsequent step to histidine residues located in a target biomolecule, which results in stable, coordinative “cross-linking” of the two molecules. Most efficient are clusters of histidine residues, as in the so-called His tag.^[19]

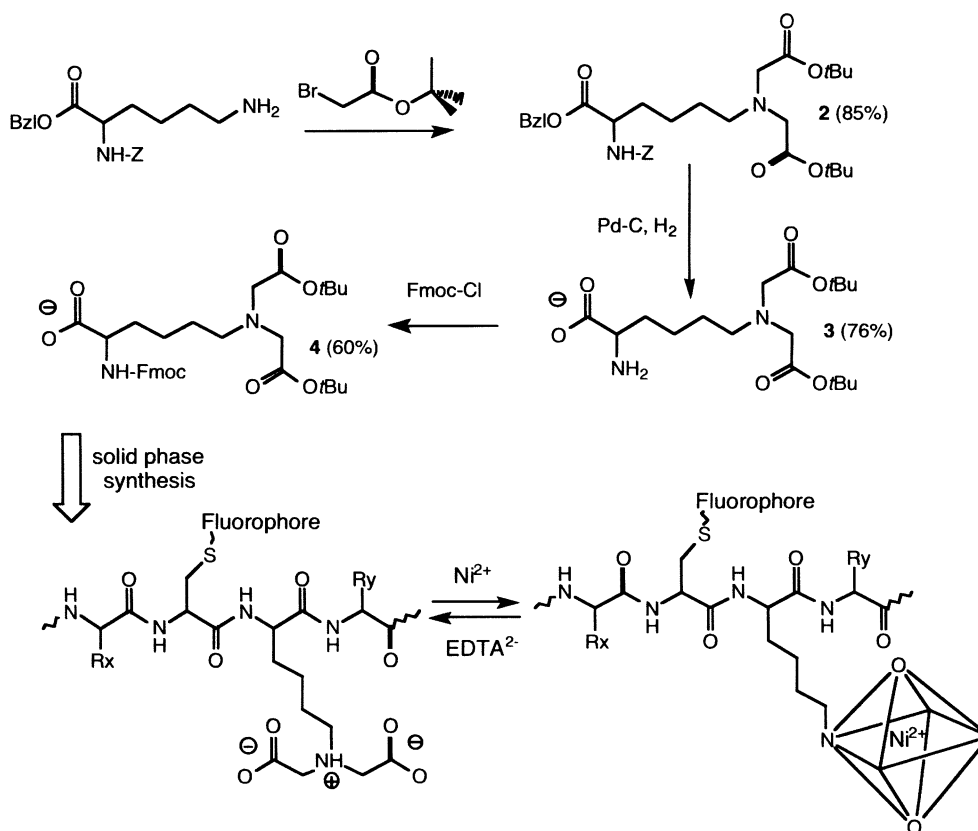
The synthesis of the metal-chelating amino acid **4** is described in Scheme 1. By starting from the commercially available L-Lys derivative N α -Z-Lys-OBzl (Z = benzyloxycarbonyl, Bzl = benzyl), Fmoc-protected IDA-Lys **4** (termed ψ) can be obtained in three steps. The protection group ensures that the building block is compatible with Fmoc solid-phase chemistry. Thus, an IDA-peptide named RRYC ψ STEL, which is derived from the antigenic epitope of human histone H3, was synthesized.

The ability of our building block ψ to form metal complexes was studied by reversed-phase (RP) HPLC analysis of the peptide RRYC ψ STEL (Figure 1). The free thiol group of the cysteine residue was blocked by iodoacetamide. The IDA-peptide eluted as a single peak at an elution time of 8.5 min (Figure 1a, black chromatogram). Preincubation of the IDA-peptide with a 10-fold molar excess of Ca^{2+} (blue chromatograph) or Mg^{2+} ions (data not shown) did not alter the elution profile, which indicates that neither calcium nor magnesium ions bind to the chelating

[a] Prof. Dr. R. Tampé, Dipl.-Chem. S. Hutschenreiter,^[+] Dr. L. Neumann,^[+] Dr. U. Rädler,^[+] Dr. L. Schmitt
Institute of Biochemistry, Biocenter
Johann-Wolfgang-Goethe University Frankfurt
Marie-Curie Straße 9, 60439 Frankfurt am Main (Germany)
Fax: (+49) 69-798-29495
E-mail: tampe@em.uni-frankfurt.de

[+] These authors contributed equally to this work.

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Scheme 1. Summary of the synthesis of the metal-chelating amino acid ψ . The 9-fluorenylmethoxycarbonyl (Fmoc)-protected metal-chelating amino acid **4** was used in solid-phase peptide synthesis to obtain fluorescein-labeled *N*_ε,*N*_ε-bis(carboxymethyl)-peptides (IDA-peptides), such as RRYC(fluorescein) ψ STEL. The coordination sphere of the metal-chelating amino acid is illustrated only schematically. Further details are given in the Experimental Section.

peptide. In contrast, incubation of the IDA-peptide with a 10-fold molar excess of Ni²⁺ yielded a new product that elutes at 9.4 min (Figure 1a, red chromatogram). The peak corresponding to uncomplexed IDA-peptide could no longer be detected. The minor second peak at 11.0 min most likely results from dimerization of the IDA-peptide through complex formation by two IDA groups, as described previously.^[20] To demonstrate that metal ions bind specifically to the building block ψ and not to the peptide backbone or any other amino acid side chain, we performed the same set of experiments with a peptide in which the metal-chelating amino acid ψ was replaced by a lysine residue. Incubation with Ni²⁺ did not change the elution profile of this IDA-free peptide (RRYC(2-acetylamide)KSTEL, Figure 1b). Next, we investigated whether IDA-peptides interact with imidazole, the simplest model ligand of the IDA group (Figure 1c and d). Incubation with Ni²⁺ ions and imidazole resulted in a change in elution time for the ψ -containing peptide, while no shift was detectable for the IDA-free peptide. These observations indicate that a specific interaction of the metal-chelating amino acid ψ with imidazole only occurs if the chelating amino acid is loaded with nickel.

Transition metal ions are able to quench a fluorophore in close proximity.^[16, 21, 22] Thus, by attaching fluorescein to the peptide adjacent to the metal-chelating unit ψ we were able to examine complex formation and interaction with ligands. Incubation of the fluorescence-labeled IDA-peptide RRYC(fluorescein) ψ STEL (50 nM) with Ni²⁺ (1 μ M) resulted in a 70–80% reduction of the

emitted fluorescence within 20 min (Figure 2a, blue) compared to the emission in the absence of Ni²⁺ (Figure 2a, black). The fluorescence response to Cu²⁺ was even faster and more pronounced (data not shown). In contrast, no change in the fluorescence spectrum was detected in the presence of 1 mM CaCl₂ (Figure 2b) or MgCl₂ (data not shown). In addition, competition experiments with the quenched, nickel-loaded IDA-peptide demonstrated that neither calcium nor magnesium ions (up to 20 mM) bind to the nickel-loaded chelating unit ψ . When the metal-chelating amino acid ψ within the peptide was replaced by a lysine residue, no fluorescence quenching was observed after addition of Ni²⁺ (Figure 2c). These data demonstrate again the specificity of complex formation between Ni²⁺/Cu²⁺ and the chelating amino acid ψ . Finally, we determined the apparent complex dissociation constant of the IDA-peptide–nickel complex by metal-induced fluorescence quenching (Figure 2d). Titration of RRYC(fluorescein) ψ STEL with nickel ions resulted in a binding curve that led to calculation of an apparent dissociation constant of 20 ± 3 nM (Figure 2d, filled squares). Again, no effect on the fluorescence emission of the IDA-peptide was detected upon titration with Ca²⁺ ions, and titration of the fluorescent IDA-free peptide with Ni²⁺ resulted in no change in emission.

The complex formed between the metal ion and the metal-chelating amino acid should be sensitive to the docking of not only imidazole and histidine but also His-tagged biomolecules. Histidine-tagged peptides have been widely used to study the

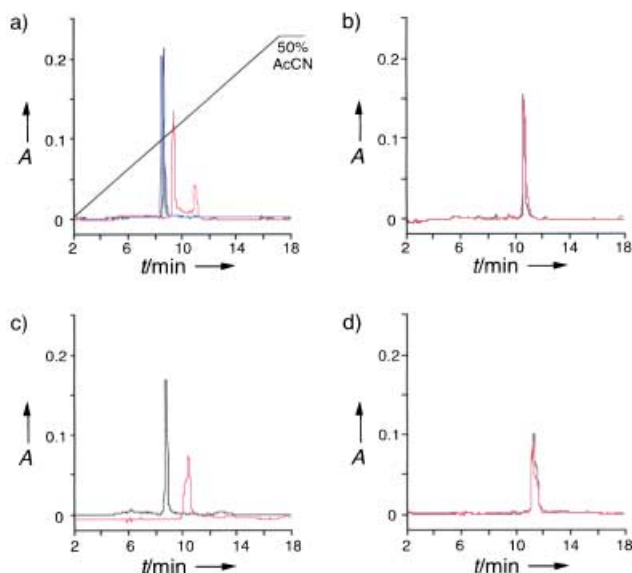


Figure 1. Specific complex formation of a metal-chelating peptide. a) RRYC(2-acetylamide)ψSTEL (50 μL, 1 mM) was incubated in the absence (black chromatogram) and in the presence of NiCl₂ (10 mM, red chromatogram) or CaCl₂ (10 mM, blue chromatogram) in buffer A and was analyzed by HPLC, with optical density recorded at 216 nm. b) RRYC(2-acetylamide)KSTEL (50 μL, 1 mM) was analyzed in the presence (red chromatogram) and absence (black chromatogram) of NiCl₂ (10 mM) in buffer A. c) RRYC(2-acetylamide)ψSTEL (50 μL, 1 mM) was incubated in the absence (black chromatogram) and presence of NiCl₂ (10 mM, red chromatogram) in buffer A^{im} (buffer A with imidazole; see the Experimental Section for details of the buffers used). d) RRYC(2-acetylamide)KSTEL (50 μL, 1 mM) was analyzed in the presence (red chromatogram) and absence (black chromatogram) of NiCl₂ (10 mM) in buffer A^{im}. In all experiments a linear gradient (0% buffer A or A^{im} to 100% buffer B or B^{im}) was applied (marked as a diagonal line in (a)).

molecular organization and orientation of histidine-tagged biomolecules at self-assembled metal-chelating interfaces.^[23–26] The IDA-peptide (50 nm) was incubated with Ni²⁺ (1 μM) and the expected fluorescence quenching was detected (shift from the black to the blue spectrum, Figure 3a). Strikingly, addition of a His-tagged peptide, (GS)₁₀H₆, resulted in an almost complete fluorescence recovery (Figure 3a, red spectrum). This recovery was comparable with the effect of imidazole (data not shown). In contrast, peptides without a histidine tag, such as (GS)₁₀ or RRYQKSTEL, did not induce any fluorescence recovery (Figure 3a, green spectrum). Control experiments with Ca²⁺ (Figure 3b) or the IDA-free peptide (Figure 3c) showed no interaction with the His-tagged peptide. Interaction between the IDA-peptide and His-tagged peptide was demonstrated by RP-HPLC (pH 7.5) and subsequent identification of both interacting partners by mass spectrometry (see Figure S1 in the Supporting Information). These data are supported by the results of fluorescence resonance energy transfer FRET studies, which provided evidence for complex formation between fluorescence-donor-labeled IDA-peptides and fluorescence-acceptor-labeled His-tagged proteins (see Figure S2 in the Supporting Information). However, we would like to stress that the magnitude of the FRET did not allow any further quantitative evaluation.

To determine the apparent dissociation constant of the peptide–peptide conjugate, increasing amounts of histidine-

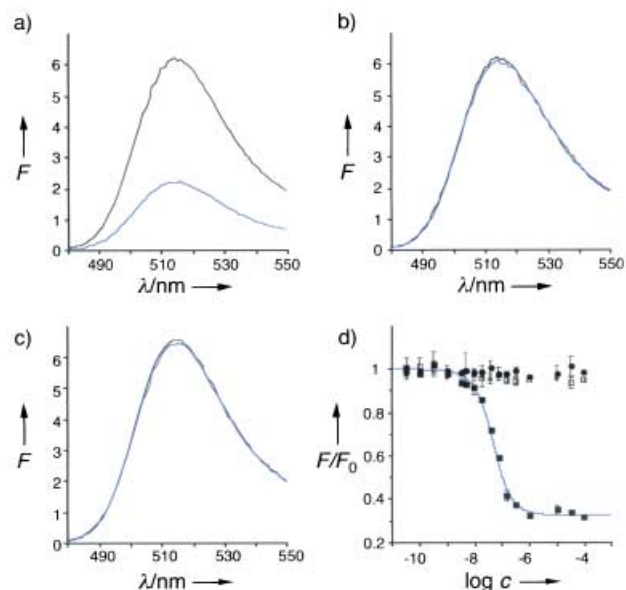


Figure 2. Sensing of nickel ions. The emission spectra of RRYC(fluorescein)ψSTEL (50 nm) in the absence (black) and presence (blue) of 1 μM NiCl₂ (a) or 1 mM CaCl₂ (b) were compared. In addition, the emission spectra of RRYC(fluorescein)KSTEL (50 nm), which lacks the IDA group, both in the absence (black) and presence (blue) of NiCl₂ (1 μM) were recorded (c). The fluorescence intensity (λ_{ex/em} = 470/535 nm) of RRYC(fluorescein)ψSTEL (50 nm) was analyzed as a function of the nickel concentration (d, filled squares). Background-corrected fluorescence intensity was normalized to the maximal fluorescence intensity F₀. As controls, experiments were carried out with Ca²⁺ (filled circles) and RRYC(fluorescein)KSTEL (open squares). All fluorescence spectra were recorded in 1 M NaCl, 40 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES), pH 7.4 at 25 °C. Error bars represent ± standard deviation (SD; n = 3). F, fluorescence intensity.

tagged peptide were titrated with the nickel-loaded IDA-peptide (Figure 3d). The obtained binding curve was analyzed as described in the Experimental Section and a dissociation constant of 96 ± 15 μM was calculated. In the absence of Ni²⁺, no change in the fluorescence spectrum was observed.

The peptides used so far represent model systems that have been designed to display weak, if any, additional electrostatic or hydrophobic interactions with the building block ψ. However, in the case of proteins such interactions must be expected and will be the rule rather than the exception. Therefore, we carried out experiments with bovine serum albumin (BSA), casein, 6 × His-tagged Mdl1-NBD,^[27] and 6 × His-tagged 20S proteasome.^[28, 29] BSA and casein are known to interact with nearly every surface or protein through hydrophobic interactions. The 20S proteasome, which plays an essential role in the homeostasis of the cell, was chosen to demonstrate that our approach is also applicable to multiprotein complexes. As summarized in Table 1, incubation of RRYC(fluorescein)ψSTEL with the His-tagged proteins resulted in a fluorescence recovery comparable to the intensity increase observed with the His-tagged peptide. In contrast, no fluorescence recovery of the Ni–IDA-peptide complex was observed after incubation with BSA or casein.

Our data demonstrate that the chelating amino acid ψ binds Ni²⁺ and Cu²⁺ ions with nanomolar affinities, but does not bind Ca²⁺ or Mg²⁺ ions. The fact that neither Ca²⁺ nor Mg²⁺ would interfere with measurements made using sensors containing ψ is

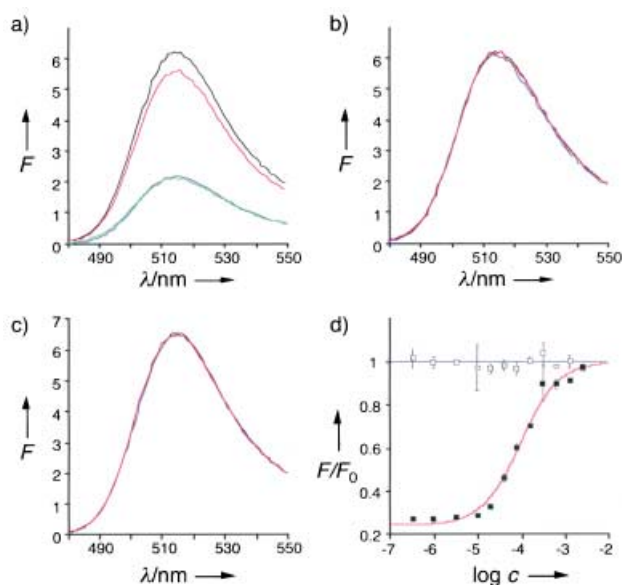


Figure 3. Sensing of histidine-tagged peptides. The emission spectra of RRYC-(fluorescein) ψ STEL (50 nm) are shown in (a) and (b) and those of RRYC-(fluorescein)KSTEL (50 nm) in (c). Spectra were recorded in the absence (black) and presence (blue) of 1 μ M NiCl₂ (a) or 1 mM CaCl₂ (b) and after addition of 1 mM His-tagged peptide, (GS)₁₀H₆ (red lines in (a–c)) and of 1 mM RRYQKSTEL as a control (green line in (a)). All fluorescence spectra were measured with the sample in 1 M NaCl, 40 mM HEPES, pH 7.4 at 25 °C. d) The dependence of fluorescence quenching ($\lambda_{\text{ex/em}} = 470/535$ nm) on the concentration of His-tagged peptide in the presence (filled squares) and absence (open squares) of 10 μ M NiCl₂. Background-corrected fluorescence signals were normalized to the maximal fluorescence F_0 . Error bars represent \pm SD ($n = 2$ or 3).

Table 1. Specific binding of nickel ions and His-tagged biomolecules to the receptor peptide. Summary of fluorescence intensity changes ($\lambda_{\text{ex/em}} = 470/535$ nm) of the IDA-peptide (RRYC-(fluorescein) ψ STEL, 50 nm) in the presence of different metal ions (1 μ M), peptides (1 mM), or proteins (0.35 μ M). All fluorescence intensities are normalized to the fluorescence intensity of the IDA-peptide in the absence of metal ions.

	Fluorescence intensity [%]
IDA-peptide	100
+ Ni ²⁺	20
+ Ca ²⁺	100
+ Mg ²⁺	100
IDA-peptide + Ni²⁺	20
+ His-tagged peptide, (GS) ₁₀ H ₆	95
+ (GS) ₁₀	20
+ RRYQKSTEL	20
IDA-peptide + Ni²⁺	20
+ His-tagged proteasome	79
+ His-tagged Mdl1-NBD	88
+ casein	20
+ BSA	19

very important for studies of proteins where the function or stability depends on the presence of Ca²⁺ or Mg²⁺ ions. As a result of the use of fluorescence as the read-out for metal-ion binding, a highly sensitive set-up was developed that needs only minimal sample concentration or volume. In terms of detection efficiency, this approach is superior to, for example, introduction of 8-hydroxyquinoline (oxine) into peptides as a sensor of metal binding.^[30] In addition, the fluorescence recovery upon binding

of His-tagged proteins might be a new and valuable tool in the detection of histidine fusion proteins, for instance, within whole-cell extracts. Control peptides or proteins without a His tag did not bind to the IDA-peptide, which indicates the specificity and selectivity of the recognition process. But even more sophisticated applications than sensors can be envisioned. Combinatorial peptide libraries containing more than one metal-chelating amino acid ψ could be used for the selection of optimal, high-affinity peptide partners to extend the concept to multivalent binding. Thus, novel high-affinity, low-molecular-weight interaction pairs, termed biochemical tweezers, could be identified. Alternatively, placement of the chelator amino acid at defined positions of a polypeptide chain by in vitro translation^[31, 32] or the intein strategy^[33–35] would generate protein–protein interfaces for switchable intra- and intermolecular interactions. Metal-chelating amino acids may further guide the design of proteins with novel properties.

Experimental Section

N α -Z-N ϵ ,N ϵ -bis(tert-butyloxycarbonylmethyl)-L-lysine-benzylester (2): tButyl bromoacetate (2.8 mL, 19 mmol) and diethylisopropylamine (DIPEA; 4.0 mL, 23 mmol) were added to a solution of N α -Z-L-lysine-benzylester (2.5 g, 4.7 mmol) in dimethylformamide (30 mL). The reaction vessel was purged with nitrogen and heated to 50 °C for 24 h with continuous stirring. The volatile compounds were removed at 50 °C under reduced pressure over 4 h. The crude product was dissolved in CHCl₃/DIPEA (50:4, 54 mL) and washed with water (3 \times 30 mL). The organic phase was dried over anhydrous sodium sulphate and the solvent removed under reduced pressure. Yield: 2.4 g (85%). TLC (CHCl₃/MeOH (10:1)): $R_f = 0.3$. ¹H NMR (400 MHz, CDCl₃/trifluoroacetic acid (TFA)): $\delta = 7.35$ (m; 5 H-Z, 5 H-Bzl), 5.20 (s; 2 H-Bzl), 5.12 (s; 2 H-Z), 4.45 (m; C2-H), 4.10 (m, 4 H; N-(CH₂)₂), 3.45 (m, 2 H; C6-H), 1.95–1.60 (brm, 4 H; C3-H, C5-H), 1.49 (s, 18 H; (CH₃)₃), 1.39 (brm, 2 H; C4-H) ppm. ¹³C NMR (125 MHz, CDCl₃/TFA): $\delta = 172.61$ (COO-Bzl), 164.99 (COO-(CH₃)₃), 135.56 (C-Bzl), 129.38 (C-Bzl), 129.16 (C-Bzl), 128.77 (C-Bzl), 86.77 (2 C; C-(CH₃)₃), 68.50 (Ar-CH₂), 56.62 (2 C; N-CH₂-COO-(CH₃)₃), 56.12 (N-CH-COOH), 54.98 (CH₂-CH₂-N-(CH₂)₂), 54.05, 32.57, 31.88 (CH₂-CH₂-N-(CH₂)₂), 28.43 (6 C; (CH₃)₃), 24.51 (CH-CH₂), 22.67 (CH-CH₂-CH₂) ppm. MS (ESI): $[M - H]^+ 599$.

N ϵ ,N ϵ -bis(tert-butyloxycarbonylmethyl)-L-lysine (3): N α -Z-N ϵ ,N ϵ -bis(tert-butyloxycarbonylmethyl)-L-lysine-benzylester (2; 1.2 g, 2 mmol) was dissolved in MeOH (100 mL) and, after the addition of 10% Pd/C, the reaction mixture was stirred under a hydrogen atmosphere at 25 °C and ambient pressure for 3 h. The catalyst was filtered off and the solvent was removed under vacuum. The crude product was purified by silica column chromatography with CHCl₃/MeOH (3:1) as eluent. Yield: 560 mg (75%). TLC (CHCl₃/MeOH/30% AcOH, 5:3:1): $R_f = 0.5$. ¹H NMR (400 MHz, CDCl₃/TFA): $\delta = 4.00$ (brm, 4 H; N-(CH₂)₂), 3.84 (t; C2-H), 3.29 (m, 2 H; C6-H), 1.90 (m, 2 H; C3-H), 1.68 (m, 2 H; C5-H), 1.45 (brm, 2 H; C4-H), 1.39 (s, 18 H; (CH₃)₃) ppm. ¹³C NMR (125 MHz, CDCl₃): $\delta = 171.40$ (COOH), 82.53 (2 C; C-(CH₃)₃), 56.39 (2 C; N-CH₂-COO-(CH₃)₃), 54.35 (N-CH-COOH), 30.48 (CH₂-CH₂-N-(CH₂)₂), 28.37 (6 C; (CH₃)₃), 26.86 (CH-CH₂), 22.97 (CH-CH₂-CH₂) ppm. MS (ESI): $[M - H]^+ = 375.1$.

N α -Fmoc-N ϵ ,N ϵ -bis(tert-butyloxycarbonylmethyl)-L-lysine (4): Fmoc-chloride (205 mg, 0.8 mmol) and DIPEA (0.14 mL, 0.8 mmol) were added in succession to a solution of N ϵ ,N ϵ -bis(tert-butyloxycarbonylmethyl)-L-lysine (3; 300 mg, 0.8 mmol) in dry CH₂Cl₂ (8 mL) and the reaction mixture was stirred for 3 h at 25 °C under a nitrogen

atmosphere. The volatile compounds were removed under reduced pressure and the crude product was redissolved in CH_2Cl_2 (20 mL) and washed with an aqueous solution of NaH_2PO_4 (100 mM, 3×5 mL, pH 5.0). The organic phase was dried over anhydrous Na_2SO_4 and the solvent removed under vacuum. The crude product was purified by silica column chromatography with $\text{CHCl}_3/\text{MeOH}$ (50:1) as eluent. Yield: 200 mg (60%). TLC ($\text{CHCl}_3/\text{MeOH}$, 5:1): $R_f = 0.8$. ^1H NMR (400 MHz, CDCl_3): $\delta = 7.75$ (d; 2H-Fmoc), 7.55 (brs; 2H-Fmoc), 7.39 (t; 2H-Fmoc), 7.29 (t; 2H-Fmoc), 4.95 (brs; C2-H), 4.40 (d; 2H-Fmoc), 4.19 (t; H-Fmoc), 4.10 (brm, 4H; N-(CH_2)₂), 3.38 (m, 2H; C6-H), 1.75 (m, 2H; C3-H), 1.61 (m, 2H; C5-H), 1.47 (s, 18H; (CH_3)₃), 1.39 (brm, 2H; C4-H) ppm. ^{13}C NMR (125 MHz, CDCl_3): $\delta = 171.48$ (COO(CH_3)₃), 163.53 (COOH), 157.05 (C=O, Fmoc), 144.68 (2C; Fmoc), 141.87 (2C; Fmoc), 128.21 (2C; Fmoc), 127.72 (2C; Fmoc), 125.60 (2C; Fmoc), 120.46 (2C; Fmoc), 82.00 (C-(CH_3)₃), 67.60 (Fmoc), 56.40 (2C; N- CH_2 -COO-(CH_3)₃), 55.57 (N-CH-COOH), 54.91 (CH_2 - CH_2 -N-(CH_2)₂), 47.86 (CH_2 - CH_2 O, Fmoc), 32.89 (CH_2 - CH_2 -N-(CH_2)₂), 32.21 (HOOC-CH- CH_2), 28.78 (6C; (CH_3)₃), 23.62 (CH- CH_2 - CH_2) ppm. MS (ESI): $[M - \text{H}]^+ = 597.5$.

Synthesis of metal-chelating peptides: Peptides were synthesized by the solid-phase technique with standard Fmoc chemistry and were labeled with fluorescein at cysteine residues as described previously.^[36] In brief, cysteine-containing peptides were incubated with a 1.2-fold molar excess of 5-iodoacetamido fluorescein (Molecular Probes) in phosphate-buffered saline, 20% (v/v) dimethylformamide, pH 6.0 for 2 h at room temperature. After purification by RP-HPLC, the identity of the peptides was verified by mass spectrometry.

BSA and casein were obtained from Sigma. *Thermoplasma acidophilum* proteasome (6 \times His-tagged at the C terminus of the β subunit) and the nucleotide-binding domain (NBD) of Mdl1 from yeast (6 \times His-tagged at the N terminus) were expressed and purified as described previously.^[27–29]

Reversed-phase HPLC: The interaction of peptides RRYC ψ STEL and RRYCKSTEL with Ni^{2+} ions and imidazole was analyzed by reversed-phase chromatography (SMART system, $\mu\text{RPC C2/C18 SC2.1/10}$, Amersham Pharmacia Biotech). Free SH groups were blocked by a 12-fold molar excess of 2-iodoacetamide (Sigma) in NaCl (150 mM), $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ (10 mM, pH 6.5). Peptides (1 mM) were incubated in buffer A (300 mM NaCl, 100 mM NH_4Ac , H_2O , pH 8.0; 50 μL) for 15 min at 25 $^\circ\text{C}$ in the presence or absence of NiCl_2 (10 mM). Subsequently, peptides were analyzed by RP-HPLC with a linear gradient of 0–100% buffer B (300 mM NaCl, 100 mM NH_4OAc , 50% acetonitrile, pH 8.0). The specific interaction of imidazole with RRYC(2-acetylamine) ψ STEL or RRYC(2-acetylamine)KSTEL was analyzed by using a gradient from buffer A^{im} to buffer B^{im} (as buffers A and B but each containing 100 mM imidazole).

Fluorescence spectroscopy: Fluorescence emission spectra ($\lambda_{\text{ex}} = 470$ nm) of fluorescein-labeled peptides (50 nM) were recorded in the absence and presence of NiCl_2 , CaCl_2 , or MgCl_2 in HEPES buffer (1 M NaCl, 40 mM HEPES, pH 7.4) at 25 $^\circ\text{C}$. Details of the fluorescence spectroscopy technique are described elsewhere.^[36] For titration experiments, RRYC(fluorescein) ψ STEL was incubated with increasing concentrations of NiCl_2 in HEPES buffer. Fluorescence signals were background-corrected. Data were normalized to the maximal fluorescence F_0 in the absence of NiCl_2 and fitted to Equation (1):

$$\frac{F}{F_0} = 1 + \frac{(E_0 + L + K_d) - \sqrt{(E_0 + L + K_d)^2 - 4E_0L}}{2E_0/\Delta F_{\text{max}}} \quad (1)$$

E_0 represents the concentration of IDA-peptide (50 nM), while L is the ligand (Ni^{2+}) concentration. ΔF_{max} represents the maximal change in fluorescence emission and K_d is the dissociation constant.

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Keywords: biosensors • fluorescence • metal-chelating peptides • protein engineering • receptors

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