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Design and Structure Determination of a Composite Zinc Finger Containing a Non-peptide Foldamer Helical Domain

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ABSTRACT

A number of foldamer backbones have been described as useful mimics of protein secondary structure elements, enabling for example the design of synthetic oligomers with the ability to engage specific protein surfaces. Synthetic folded backbones can also be used to create artificial proteins in which a folded peptide segment (e.g. an α -helix, a loop) is replaced by its unnatural counterpart, with the expectation that the resulting molecule would maintain its ability to fold while manifesting new exploitable features. The similarities in screw sense, pitch and polarity between peptide α -helices and oligourea 2.5-helices suggest that a tertiary structure could be retained when swapping the two backbones in a protein sequence. In the present work we move a step towards the creation of such composite proteins by replacing the 10-residue long original α -helical segment in the Cys2His2 zinc finger 3 of transcription factor Egr1 (also known as Zif268) by an oligourea sequence bearing two appropriately spaced imidazole side chains for zinc coordination. We show by spectroscopic techniques and mass spectrometry analysis under native conditions that the ability of the peptide/oligourea hybrid to coordinate zinc ion is not affected by the foldamer replacement. Moreover, detailed NMR analysis provides evidence that the engineered zinc finger motif adopts a folded structure in which the native beta-sheet arrangement of the peptide region and global arrangement of DNA binding side chains are preserved. Titration in the presence of Egr1 target DNA sequence supports binding to GC bases as reported for the wild type zinc finger.

Keywords: Zinc finger, oligourea foldamer, NMR structure, protein prosthesis, helix mimicry

INTRODUCTION

Recent progress in the creation of artificial proteins with accurate control over chemical composition, three-dimensional structure, and appending functionalities¹⁻⁶ open avenues for a range of downstream applications from therapeutics and delivery systems to catalysts and bionanomaterials. In this context, protein backbone engineering (protein prosthesis⁷),³ which benefits from current advances in the total chemical synthesis and semi-synthesis of proteins,⁸⁻¹² can be used to interrogate protein folding, address the role of individual folded segments, modulate protein function and fine tune a range of properties among which immunogenicity, solubility, resistance to proteolytic degradation and cell permeability. A number of reports have investigated the effect of substituting the amide bond by surrogates, or small subsets of α -residues in loop and turn segments by either constrained or more flexible mimics.^{7,10,13-21} Fewer studies, however, have reported composite proteins in which a larger folded segment (e.g. an α -helix) was replaced by an artificial one. The ability to create isolated and stable artificial secondary structure elements from a variety of non-biological synthetic oligomeric backbones (*i.e.* foldamers²²⁻²⁴) either close or remote from natural biopolymers provides new opportunities to design and investigate such composite proteins.²⁵ A noteworthy example is a semisynthetic analogue of human interleukin 8 (IL-8) in which the C-terminal α -helix was replaced by a 14-residue long β^3 -peptide sequence.²⁶ Although the artificial chemokine retained significant biological activity, it should be noted that the incorporated 3_{14} -helical β -peptide segment differs from the original α -helix in both handedness and dipole orientation.^{26,27} In the absence of high-resolution structural information, it is not possible to draw firm conclusion about the level of mimicry that was actually achieved in this system in terms of tertiary structure. Concurrently, structural details about the effect of multiple β -residue substitutions evenly distributed in α -helical segments of discrete tertiary structures (e.g., B1 domain of protein G from *Streptococcus bacteria* (GB1),²⁸ villin headpiece subdomain,²⁹) and within coiled coils (e.g., yeast transcriptional activator GCN4-derived peptides)³⁰⁻³² have been reported independently by Gellman and Horne. In particular, the Horne group has shown that the GB1 tertiary fold can accommodate up to 20% unnatural residues (*i.e.*, a combination of D-amino acid, aminoisobutyric acid, N-Me-amino acid, and β^3 -amino acid replacements) strategically placed in different secondary structure elements (helix, loop, sheet, and turn)²⁸ highlighting the substantial

plasticity of the protein backbone.

Swapping a whole secondary structure element such as an α -helix with its foldamer counterpart in a protein context without causing major unfolding remains a challenge and ideally requires the two folded backbones to exhibit a high degree of similarity and complementarity in terms of shape and side chain distribution. We have previously reported that aliphatic N,N'-linked oligoureas, a class of non-peptidic foldamers that can be decorated with proteinogenic side chains, adopt a helical conformation closely resembling the α -helix in terms of diameter, pitch, handedness, and polarity.³³ Their stability in water and their high tunability have already been exploited to program the formation of complex assembled architectures, such as discrete helical bundles and channels.³⁴⁻³⁶ Furthermore, it has been shown in model chimeric molecules consisting of fused oligourea and α -helical peptides segments (so called block co-foldamers), that the two helical structures both co-exist and communicate.³⁷⁻³⁹ Although the canonical oligourea and peptide helices differ by the number of residues per helix turn (2.5 for oligoureas³³ versus 3.6 for α -peptides), an overlay of the two helical backbone reveals a relatively close match between a three-residue-long oligourea and an α -helical tetrapeptide (Root-mean square deviations (RMSDs) calculated for four carbonyl C atoms and four NHs = 0.7867 Å, Fig. 1a). This observation suggested to us that a composite foldamer protein could be generated by substituting an α -helical segment for a designed oligourea sequence of appropriate length, although care must be taken to preserve the distribution and space orientation of key side chains.

To test our ability to design such a foldamer-based mimic of a folded protein, we selected the Cys2His2 zinc finger motif. Zinc fingers are recurrent protein motifs (found in 2% of human genes) involved in specific duplex DNA recognition, whose folding properties have been extensively studied using native and simplified synthetic systems.⁴⁰⁻⁴³ Cys2His2 zinc fingers are (relatively) small and modular units of about 30 residues, which consist of two antiparallel strands of β -sheet connected by a turn, packed against an α -helix. This archetypal $\beta\beta\alpha$ fold is stabilized by a single zinc ion coordinated by a pair of cysteine residues located in the β -sheet and a pair of histidine residues located at the C-terminus of the α -helix in a tetrahedral geometry (Fig. 1b)⁴² Zinc coordination is playing a key role at directing folding of the motif and at correctly orienting α -helix residues in direct contact with DNA, and thus it is crucial that the histidine and cysteine side chains

are properly displayed. To the best of our knowledge, only few studies have investigated the effect of side chain metal coordination for the stabilization of foldamer secondary⁴⁴⁻⁴⁹ or tertiary structures.⁵⁰ Here, we selected Egr1 (Zif268), a transcription factor involved in cell proliferation and in the regulation of apoptosis as a target zinc finger protein. We describe the design and synthesis of a chimeric oligoureia-zinc finger mimic whereby the native α -helically folded segment containing the two key histidines and the DNA –binding side chains has been replaced by an oligoureia sequence (Fig. 1b).⁵¹⁻⁵² We have studied in detail the metal binding properties of the resulting composite protein by UV-Vis spectroscopy, circular dichroism (CD), ESI-MS and NMR analyses and have determined its three-dimensional solution structure by NMR spectroscopy. Our results show that we successfully conceived a composite zinc finger that mimic the native protein in different aspects such as folding, metal and DNA binding.

RESULTS AND DISCUSSION

Design and synthesis. We focused our efforts on one of the three zinc finger motifs of the zinc finger region (hereafter numbered from residue 1 to 90) of Egr1, namely finger 3 (Fig. 1b) encompassing residues 62-90. Examination of the X-ray crystal structure of a complex between a protein fragment encompassing the three fingers of Egr1 and a duplex DNA with a consensus binding site,^{53,54} revealed that the α -helix domain of finger 3 spans 11 residues (three helical turns) from Ser75 to His85. An overlay of the zinc finger helix with the X-ray crystal structure of a canonical oligoureia helix³⁴ suggested to us that the distribution and spacing of the two key imidazole side chains (His81 and His85) could be preserved by substituting a short oligoureia segment of 8 residues (whose sequence is shown in Fig. 1c) for the residues Asp76-His85 of the α -helix. His-type side chains which are in a $i/i+4$ relationship in the natural peptide sequence, are in a $i/i+3$ relationship in the chimeric molecule. It is noteworthy that the side chains at the N-terminus of the α -helix known to contact nucleobases in the major groove of DNA, namely Asp76 at P2, Glu77 at P3 and Arg80 at P6 have been maintained in the sequence of the cognate oligoureia foldamer segment. The artificial zinc finger ^{urea}**Fn3** was assembled using a fragment condensation approach on solid support, taking advantage of the presence of a glycine residue in the sequence (Fig. 2a, supplementary methods).

The hybrid C-terminus which contains the oligourea segment was synthesized on MBHA resin by microwave assisted solid-phase synthesis (SPS) using a set of N-Boc-protected-1,3-diamino monomers carrying the side chains of Ile, Lys, Asp, Glu and His (**BB1-BB6**) activated as succinimidyl carbamates for urea formation^{55,56} along with standard N-Boc α -amino acids. The key monomer containing the (1*H*-imidazolyl-4yl)methyl side chain of histidine (**BB6**) was prepared from Trt-His(τ -Trt)-OMe in seven steps with an overall yield of 35 % as described previously.⁵⁶ The fully protected N-terminal fragment (residues 62-68) was synthesized on 2-chlorotrityl resin using N-Fmoc α -amino acids (plus Boc-Pro-OH as last residue) and released by treatment with a 3:2 mixture of hexafluoroisopropanol (HFIP) and CH₂Cl₂⁵⁷. It was then reacted directly with the α -amino function of the solid-supported C-terminal fragment in the presence of BOP and HOBt as coupling agents, to afford the desired ^{urea}**Fn3** with a 68% crude yield after HF cleavage. For head to head comparison, we also synthesized the native finger 3 peptide (^{native}**Fn3**) using standard SPS methodology and Fmoc strategy (see Supporting Information for details). Both ^{urea}**Fn3** and ^{native}**Fn3** were purified by preparative reversed-phase HPLC (C₁₈) to a final purity of >95%, lyophilized and characterized by ESI-MS (Fig. 2b).

Metal binding properties of ^{urea}Fn3**.** The ability to coordinate Zn²⁺ cannot be directly assessed by UV-Vis spectroscopy, because Zn²⁺ has a full d¹⁰ shell, and is therefore UV silent. We thus performed a first titration experiment using Co²⁺, which is known to form complexes with the same geometry as Zn²⁺ and has a partially empty d-shell. A signal between 500 and 800 nm, characteristic of the d-d transition of a tetrahedral Co²⁺ complex was observed upon titrating both ^{urea}**Fn3** and ^{native}**Fn3** with CoCl₂ (Fig. 3a and Supplementary Information).

A sharp end point was reached in both cases upon addition of 1 equivalent of metal, supporting a 1:1 stoichiometry for the complexes and strong binding. The zinc finger–Co²⁺ complexes were subsequently retro-titrated with ZnCl₂, and for both compounds, a decrease of the signal in the visible region was observed, indicating a displacement of Co²⁺ by Zn²⁺ in the complex (Fig. 3b and Supplementary Information). The stability of the two zinc finger–Zn²⁺ complexes was estimated by fitting the titration data to a 1:1 competitive binding model and very similar *K_d* values were obtained (*K_d* = 2·10⁻⁹ M for ^{urea}**Fn3**–Zn²⁺ and 6·10⁻⁹ M for ^{native}**Fn3**–Zn²⁺). In contrast, the kinetics of Co²⁺ displacement varied between the two zinc fingers from 4 minutes for ^{urea}**Fn3**, to 20 minutes for

^{native}**Fn3** (see Supplementary Information). Since the end point of the direct titration with Co^{2+} was too sharp, the K_d of the zinc finger– Co^{2+} complexes was determined in an independent UV titration experiment, by using CoCl_2 in the presence of NTA as competitor ($K_d \text{ NTA–Co}^{2+} = 8.1 \cdot 10^{-8} \text{ M}$,⁵⁸ see Supplementary Information).⁴³

To gain additional insight into the effect of metal complexation on the overall tertiary structure, we also recorded CD spectra for the two Zn finger motifs at increasing concentrations of Zn^{2+} . For ^{native}**Fn3** a decrease in the negative molar ellipticity around 200 nm and an increase of the signal around 220 nm are observed upon ZnCl_2 addition. A similar behavior was described in previous reports^{59,60} and it is characteristic for the $\beta\beta\alpha$ arrangement of a zinc finger motif. The CD signature of ^{urea}**Fn3** differs substantially from the native domain: the molar ellipticity is positive around 200 nm, which is attributable to oligourea 2.5-helical folding. Furthermore, a negative signal at 230 nm, which decreases upon ZnCl_2 addition, suggests a partial reorganization of the system upon metal binding (Fig. 3c and supplementary Fig S8). The titration end point was reached upon addition of 1 equivalent of the metal in agreement with UV-vis spectroscopy experiments and the formation of a 1:1 complex. The stoichiometry of the complex with Zn^{2+} was further supported by native mass spectrometry experiments (Fig.4). For both motifs, only the 1:1 complexes were observed in the presence ZnAc_2 (3+ and 4+ charge states being the most populated regardless of the amount of metal added).

To further examine the metal binding profile of ^{urea}**Fn3** in comparison with ^{native}**Fn3**, we recorded ESI-MS spectra in the presence of equimolar amounts of Zn^{2+} , Co^{2+} , Cd^{2+} and Hg^{2+} and an excess of zinc finger in 100 mM NH_4OAc , pH ~6.8 (Fig. 4b). By comparing the relative intensities of the species observed it was possible to estimate the relative stability of the complexes formed, and thus the metal binding preferences of each motif. However, since the affinity of Hg^{2+} for NH_3 significantly differs from that of the other metal ions, Hg^{2+} being the most sequestered by NH_3 (from NH_4Ac), care must be taken when interpreting intensity ratios with zinc finger– Hg^{2+} complexes. The 1:1 complex with Zn^{2+} and the 1:1 complex with Co^{2+} were the most abundant species detected with both native and urea-containing sequences. The two sequences were found to differ by their binding profile to heavy metals. Whereas only a weak signal corresponding to 1:1 complexes with Cd^{2+} and Hg^{2+} was observed for the native sequence, the chimeric motif was found to readily bind

Cd²⁺ and Hg²⁺ as revealed by the intense peaks corresponding to the 1:1 complexes and also by the substantial amount of the 2:1 ^{urea}**Fn3**—Cd²⁺ complex in the ESI-MS spectrum.

¹H-NMR analysis qualitatively supports folding of ^{urea}Fn3 upon Zinc coordination. One-dimensional ¹H NMR spectra of ^{urea}**Fn3** were recorded in the absence and presence of one equivalent of Zn²⁺ metal ions in 10 mM Sodium phosphate buffer (NaPi) buffer at pH 6.5. ¹H NMR spectral comparison, depicted in Fig. 5a show remarkable changes in the chemical shift dispersion upon zinc addition indicating structural fluctuation and conformational change. In the absence of zinc ¹H NMR spectra of ^{urea}**Fn3** show broader resonance lines and limited chemical shifts dispersion of NH (both amide and urea) signals, suggesting the absence of well-defined structure. Conversely, in the presence of zinc, the ¹H NMR spectra of ^{urea}**Fn3** feature increased chemical shift dispersion of NH signals and sharp resonance peaks supporting the formation of a folded conformation as a result of metal coordination.

NOE analysis and structure calculation. Sequence-specific assignment of the 1:1 ^{urea}**Fn3**—Zn complex was achieved using multiple multidimensional homonuclear and heteronuclear NMR experiments including 2D DQF-COSY, ¹H-¹⁵N HSQC, ¹H-¹³C edited HSQC, ¹H-¹H TOCSY and ¹H-¹H NOESY. High resolution 2D ¹H-¹H NOESY spectra obtained at high field (950 MHz) were necessary to unambiguously assign and sequentially link each oligourea spin system. In the end, nearly 97% of the expected resonances for ^{urea}**Fn3**—Zn were obtained (Fig. 5b, Supplementary Table S1).

Qualitative inspection of inter-residue NOEs extracted from NOESY experiments at 950 MHz revealed several key features supporting folding of ^{urea}**Fn3**—Zn into a defined tertiary structure. Firstly, the presence of repeated and typical medium range NOE cross peaks of the type H α (*i*)/HN(*i*+2) and H α (*i*)/HN²(*i*+2) all along the oligourea segment is consistent with the formation of a canonical oligourea 2.5-helix as previously reported.^{61,62} A number of characteristic inter-residue NOEs are also observed in the peptide region encompassing residues 62-75. In addition to NOE cross peaks between side chain protons : Phe72 H δ 1 / Cys65 H β and Cys65 H β / Cys68 H α , long range NOEs between Phe72 HN and Ala64 H α , Phe63 HN and Ala64 H α , Asp66 H α and Gly69 HN, and Ile67 HN and Gly69 HN support the presence of a β -sheet structure. The long range NOEs observed between side chains of the β -sheet region and the oligourea helix/C-terminal

tripeptide, such as those between H β , H γ and H δ of Ile67 and protons H δ 1 and H ϵ 1 from His^U83, suggest that both parts are aligned side-by-side. Moreover, the following NOE cross peaks Cys65 H β / Cys68 H β , Cys65 H β / His^U80 H β , and Cys68 H β / His^U83 H β indicate that the side chains of these ligand residues are in close spatial arrangement to satisfy zinc metal ion binding. The presence of NOE intra-residue crosspeaks between imidazole H ϵ 1 with H δ 1 protons in His^U80 and His^U83 residues confirmed protonation at the N δ position and that the N ϵ position is free to coordinate zinc metal ion. The solution NMR structure of the 3.4 KDa ^{urea}**Fn3**—Zn was calculated using 231 experimental NOE inter proton distances in combination with 22 torsional angles (for α -L amino acids) and 32 weak hydrogen bond restraints for both natural and urea-type residues (Table S1) as described in the supplementary information. Briefly, NOE inter proton distance restraints were extracted from the in-depth analysis of 2D NOESY spectra acquired at different mixing time 120 and 250 ms and used for structure calculation in a simulated annealing protocol with AMBER 12 molecular dynamics package.⁶³ The 200 best lower energy coordinates from vacuum simulation were selected for further minimization and 40 ps simulated annealing NMR refinement using generalized Bohr (GB) implicit solvent model. The 10 conformers with the lowest total energy were extracted from the MD trajectory and subjected to further refinement in an explicit water box with periodic boundary condition. The final structure ensemble is shown in Fig. 6a and structural statistics are given in Table S1.

Owing to the composite nature of ^{urea}**Fn3**, the final NMR ensemble of conformers could not simply be checked with online structure validation tools. Structures of ^{urea}**Fn3** were validated in two steps by first analyzing dihedral angles in the C-terminal oligourea segment over 40 ps of restrained molecular dynamics in comparison with values extracted from X-ray crystal structures of oligourea helices.^{33,34} In a second step, the α -peptide segment in the structure (*i.e.* residues 62-75) was submitted to PROCHECK⁶⁴ for validation.

Solution structure of ^{urea}Fn3** and comparison with the native fold.** The solution structure of ^{urea}**Fn3**—Zn was obtained with a well-defined backbone core and a root-mean-square deviation (RMSD) of 0.35 Å. As shown in Fig. 6, ^{urea}**Fn3** adopts a native-like tertiary structure consisting of a short antiparallel β sheet connected to the helically folded oligourea segment. The structure is held

together by tetrahedral zinc coordination of the two Cys residues in the peptide region and the imidazole side chains of the two His^U units in the 2.5-helix oligourea segment. A general feature of native $\beta\beta\alpha$ zinc fingers is the presence of a small hydrophobic core that brings additional stabilization to the structure and that generally involves a highly conserved aromatic residue (Phe72 in ^{native}**Fn3**) packed edge to face against the proximal histidine (His81 in ^{native}**Fn3**). Compared to the $\beta\beta\alpha$ fold of ^{native}**Fn3** (PDB ID: 1AAY),⁵⁴ the hydrophobic core in ^{urea}**Fn3** is less compact because the loop connection between the β -strand and the helix region is more extended. This distortion of the loop segment may be due in part to the larger diameter of the oligourea helix compared to the α -helix. However, the packing of the Phe72 over His^U80 is maintained. Conformational similarities and differences between ^{native}**Fn3** and ^{urea}**Fn3** are further highlighted by the structural alignment of the two N-terminal peptide segments (13 to 13 C α atoms) which gives an average root-mean-square deviation of 1.166 Å (calculated for the 10 lowest-energy structures (Fig. 6d)).

DNA binding properties of the chimeric ^{urea}**Fn3** zinc finger

We next evaluated the ability of ^{urea}**Fn3** to interact with a short double-strand DNA sequence using chemical-shift mapping experiments and the Egr1 GC rich binding motif. First, the DNA imino region peak dispersion was monitored by 1D ¹H NMR spectroscopy upon successive additions of 0.5-2.5 molar equivalents of ^{urea}**Fn3** to DNA. As depicted in Fig. 7, a clear shift of several imino peaks was observed, notably G2, G4 and a third ambiguous G imino peak. ¹H-¹H NOESY experiments were used to tentatively assign imino peaks in the dense region between 12.7 and 12.9 ppm. The results seem to indicate that ^{urea}**Fn3** binds to the 5' side of the leading strand DNA duplex d(5'-¹AGCGTGGGCG¹¹T-3') and d(3'-¹TCGCACCCGC¹¹A-5'). No important chemical shift variations were observed in the 3' region of the leading strand d(5'-¹AGCGTGGGCG¹¹T-3'). Apparent dissociation constants (K_d) were obtained by fitting the titration data to a single-site ligand-binding mode and were found to be in the range of 0.19-0.25 mM (see the Supplementary Information and supplementary Fig. S9 for more details). These values are consistent with previous reports showing that a single Cys2His2 zinc finger domains bind with low affinity to DNA.⁶⁵ High affinity recognition of DNA requires cooperativity and is achieved with protein domains

containing three or more zinc fingers.

CONCLUSIONS

We have shown here that the structural resemblance between urea-based helical foldamers and α -helical peptides can be exploited by combining the two backbones in a single chain to create protein mimics with defined tertiary structures. The design of the chimeric Cys2His2-type zinc finger reported in this work was guided by the knowledge of the structures of oligourea 2.5-helices at atomic resolution and was facilitated by their predictability, the helix folding propensity of urea oligomers being largely unaffected by the nature of the side-chains used.³⁴ We observed that the metal binding ability of the composite zinc finger (^{urea}**Fn3**) was fully retained upon substituting the α -helix by the designed oligourea prosthesis, which indicates that the distribution of histidine-type side chains at the surface of the oligourea helix is fully compatible with the formation of a tetrahedral zinc complex. Furthermore, detailed NMR-derived 3D structure determination of the composite zinc finger in solution showed that the native beta-sheet arrangement of the peptide region was not compromised by helix swapping, and that a well-defined tertiary structure akin to the native fold was formed. Specific features of ^{urea}**Fn3** such as an extended loop between the β -strand and the helix region and a less compact hydrophobic core compared to ^{native}**Fn3** may account for the differences in kinetics of metal exchange and binding selectivity observed between ^{urea}**Fn3** and ^{native}**Fn3**. Despite these differences, detailed inspection of the structure also revealed that overall the relative orientation of side chains involved in DNA binding by Cys2His2 Zinc fingers (namely Arg^U74, Asp^U76, Glu^U77 and Arg^U79 corresponding to positions -1, 2, 3 and 6 of the α -helix) is relatively similar to that in the native zinc finger. The finding that ^{urea}**Fn3** binds DNA suggests that affinity could be increased significantly by incorporating it into a larger protein sequence containing multiple zinc finger motifs. It would then be tempting to evaluate how the foldamer replacement affects sequence specificity of DNA binding knowing that a given zinc finger motif is contacting three base pairs in a sequence dependent manner.

The approach reported in this work may prove to be more general and could be applied to other systems that fold without metals. The ability to combine synthetic oligourea foldamers and natural polypeptides to re-design proteins can be seen as a promising approach to create

proteinomorphous objects with properties and possible applications beyond those known for natural proteins.

EXPERIMENTAL SECTION

Chemistry. The composite zinc finger ^{urea}**Fn3** was synthesized using a fragment condensation approach by coupling the fully protected peptide encompassing residues 62-69 directly to the growing peptide/oligourea chain (residues 70-86) on the solid support. The 62-69 peptide segment was synthesized on a 2-chlorotrityl chloride resin using standard *N*-Fmoc chemistry (at the exception of the final Pro residue incorporated as *N*-Boc-Pro-OH), whereas the chimera was assembled by using *N*-Boc protected amino acid residues and *N*-Boc-protected succinimidyl carbamate building blocks for urea formation starting from a low-loading MBHA resin. The native zinc finger was synthesized on solid support by standard Solid-phase peptide synthesis using *N*-Fmoc-protected α -amino acid derivatives on low-loading Rink-amide MBHA resin. Additional details about chemical synthesis and purification and characterization of ^{urea}**Fn3** and intermediates can be found in the Supplementary Information.

UV-vis and CD spectroscopy: All the samples were dissolved in phosphate buffer 20 mM pH 7.5 and the measurements were performed under a positive atmosphere of Ar. The titrations were carried out in the presence of an excess of TCEP (about 0.5 mM), to ensure the complete reduction of the Cys residues. All the solutions used were previously purified through Chelex resin column (Bio-Rad), to eliminate any undesired metal contaminations, and degassed prior to use. For the elaboration of the data the dilution was taken into account and all the curves were corrected for the blank and the baseline adjusted to zero. The curves were fitted to the appropriate equilibrium using the software Origin 8.0. CD experiments were performed on a Jasco J-815 spectrometer. Data were recorded at 20 °C with a speed of 50 nm/min between wavelengths of 200 and 300 nm, at 1 nm intervals and with an integration time of 2 seconds. Each measurement was repeated twice and averaged. For the elaboration of the data the dilution was taken into account and all the curves were corrected for the blank and the baseline adjusted to zero. Experimental details are provided in Supplementary Information.

NMR spectroscopy, sequence specific assignment and restraints: Initial NMR experiments were performed at 298 K, unless specified otherwise, using Bruker 700 MHz, 800 MHz NMR spectrometers equipped with 5mm TXI and cryoprobe respectively, with the ability to apply pulse field gradient along z-axis. To obtain the sequence-specific-assignment of ^{urea}**Fn3**, a set of standard homonuclear NMR experiments including 2D ¹H-¹H DQF-COSY, 2D ¹H-¹H TOCSY (80 and 120 ms mixing time) and 2D ¹H-¹H NOESY (100, 150 and 300 ms mixing time) were recorded in solution contain 90% H₂O, 10% D₂O, NaOH + HCl buffer (pH 6.5, 220 μL) under physiological condition. TOCSY spin systems were sequentially interlinked using sequential NOESY cross peak. With the help of sequence specific assignment, all inter-residue NOE distance restraints for exchangeable protons were obtained from 2D homonuclear ¹H-¹H NOESY spectra acquired with mixing times of 120 and 250 ms. To unambiguously assign non exchangeable protons, 2D ¹H-¹H NOESY experiments (mixing time of 250 ms) were performed on 800 MHz using the same sample after lyophilization and re-hydration in 100% D₂O with the same final volume of 220 μL. To complement the homonuclear sequence-specific-assignment, a set of heteronuclear NMR experiments including 2D ¹H-¹⁵N, ¹H-¹³C HSQC and ¹H-¹³C NOESY experiments were performed on the same sample. Because of the presence of two amide protons (¹H^{N1-15}N₁ & ¹H^{N2-15}N₂) in each urea units, both TOCSY and NOESY spectra contain the signals of two degenerated side chain (-R, Fig. 1a) frequencies that resonate over 1.3 ppm (between 5.6 ppm to 6.9 ppm) on F2 dimension. The number of oligourea amide protons together with side chain chemical shifts degeneracy (on F1 & F2 dimensions) significantly contributed to poor spectral resolution and hampered the quality of NOESY spectra acquired on 800 MHz spectrometer. To alleviate resonance crowding and to improve both resolution and quality of NMR spectra all the above listed experiments were repeated on the same sample (90% H₂O, 10% D₂O, NaOH + HCl buffer, pH 6.5, total 220 μL) on a 950 MHz spectrometer equipped with a 5 mm cryogenically cooled probe-head with the z-axis pulse filed gradient (CERM, Florence). In all the experiments recycle delay was set to 1.5 s and water suppression was achieved by excitation sculpting method. Initial acquisition of ¹H-¹⁵N spectra with standard acquisition parameters (spectral width and offset) did not show all the expected peaks. Preliminary observation confirmed that oligourea amide proton ¹H^{N2} and its directly bonded nitrogen ¹⁵N₂ resonate far from the regular ¹⁵N/ ¹H^N chemical shift range, hence in

2D ^1H - ^{15}N NMR experiment F1 dimension(^{15}N) spectral offset was set to 100 ppm with the total spectra width of 60ppm. For the rest of the listed NMR experiments, standard offset and spectral width were used. All NMR spectra were processed using topspin 3.2 (Bruker BioSpin) and SPARKY⁶⁶ for spectra analysis and data interpretation.

Structure calculation and refinement in explicit solvent. NMR restrained based structure calculation of ^{urea}**Fn3** was performed using sander module of AMBER 12 (AMBER tool 14) and ff99SB force field⁶⁷ by distance geometry simulated annealing in GB model and restrained molecular dynamics refinement in explicit solvent as described in Supplementary Methods.

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ASSOCIATED CONTENT

The Supporting Information is available free of charge on the ACS Publications website at DOI:

Characterization data of all compounds, spectroscopy experiments and supplementary methods, Figures S1–S9 and Table S1 (PDF).

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Figure 2. Synthesis of ^{urea}Fn3. **a**, General Scheme illustrating the fragment condensation approach on solid-support which was used to assemble the composite zinc finger. **b**, HPLC trace and mass spectrometry data of the purified oligomer. Note: for clarity reasons all the side-chain protecting groups have been omitted.

Figure 3. Spectroscopic analysis of Zn^{2+} binding to ureaFn3 . **a**, UV-Vis titration of ureaFn with excess CoCl_2 . The Inset on the left present a zoom on the regions corresponding to the LMCT bands and d-d transitions indicative of a tetrahedral coordination of Co^{2+} . The inset on the right shows the concentration dependent increase of the signal upon addition of Co^{2+} . **b**, Progressive disappearance of this signature at 650 nm upon competitive titration with Zn^{2+} and curve fitting using a 1:1 binding model. **c**, CD signature of ureaFn3 and nativeFn3 in the absence and upon addition of one equivalent of Zn^{2+} .

Figure 5. ^1H NMR analyses of $^{\text{urea}}\text{FN3}$. **a**, Comparison of 1D ^1H NMR spectra of $^{\text{urea}}\text{FN3}$ in the absence (top) and in the presence of zinc cation (bottom). **b**, HN HSQC spectra of $^{\text{urea}}\text{FN3}$ (left) H^{N} from the regular amide protons and (right) H^{N} and H^{N2} from the oligourea amide protons. Oligourea residues (76-83) are colored in orange whereas regular α -amino acid residues are shown in blue. **c**, 2D ^1H - ^1H TOCSY (green) and NOESY (black) NMR spectra with a mixing time of 120 and 250 ms respectively.

Figure 6. NMR-derived model of ^{urea}FN3. **a**, ten superimposed lowest-energy structures from the **restrained molecular dynamics** rMD-GB in explicit solvent with boundary conditions. The backbone CA RMSD between inter model is 0.6-0.7 Å. Carbon atoms in the peptide segment are depicted in light blue whereas those in the oligourea segment are depicted in orange. **b**, view of a representative conformation of ^{urea}FN3 highlighting coordination to zinc metal ion. **c**, Comparison with the structure of ^{native}FN3 as found in the X-ray crystal structure of Egr1 complexed with a duplex DNA sequence (PDB ID: 1AAY⁵⁴). **d**, Structural alignment of the N-terminal peptide segments (13 Cα) of ^{urea}FN3 (one representative low energy structure) and ^{native}FN3 (PDB ID: 1AAY).

Figure 7. ¹H NMR titration of Egr1 duplex DNA d(5'-AGCGTGGGCGT-3') and d(3'-TCGCACCCGCA-5') by ^{urea}**FN3**. **a**, Part of the 800 MHz 1D ¹H NMR spectra of duplex DNA in the absence (i), and in presence of 0.5 equiv. (ii), 1 equiv. (iii), 1.5 equiv. (iv), 2 equiv. (v) and 2.5 (vi) of ^{urea}**FN3**. The addition of increasing amount of ^{urea}**FN3** caused large chemical shift changes of mainly three imino protons of DNA duplex, G2, G4 and an ambiguous imino marked with an asterisk. **b**, Zoomed region of the ¹H-¹H NOESY spectra showing chemical shift variation cross-peaks of H8 from C bases and imino protons of G bases upon addition of 2.5 molar equivalents of ^{urea}**FN3**.

TOC graphic