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ARTICLE TYPE

# Peptide-DNA conjugates as tailored bivalent binders of the oncoprotein c-Jun

Elena Pazos, Cecilia Portela, Cristina Penas, M. Eugenio Vázquez and José L. Mascareñas\*<sup>a</sup>

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We describe a ds-oligonucleotide-peptide conjugate that is able to efficiently dismount preformed DNA complexes of the bZIP regions of oncoproteins c-Fos and c-Jun (AP-1), and therefore might be useful as disrupters of AP-1-mediated gene expression pathways.

## Introduction

Gene expression is a highly regulated process that involves the interaction of proteins called transcription factors (TFs) with specific regulatory sites of the genome.<sup>1</sup> The dysfunctional activity of TFs can lead to errors in the expression of specific gene products, and eventually to anomalous behaviour of the cells.<sup>2</sup> When TFs are involved in the regulation of cell proliferation events, such as in the case the oncoprotein c-Jun, this irregular behaviour might result in uncontrolled cell proliferation and cancer.<sup>3</sup> In fact, there is a large number of evidences on the presence of unusual levels of c-Jun in different types of human cancers, which suggest that this protein plays a role in the malignant transformation of cells.<sup>4</sup> Therefore, there is a high interest on the development of strategies that enable to control the activity of this transcription factor.<sup>5</sup>

The mechanism of action of c-Jun involves the formation of a coiled-coil heterodimer with other bZip protein components, prominently with c-Fos, to form the so-called AP-1 complex with specific DNA sites (5'-ATGACTCAT-3').<sup>6</sup> Earlier reports using this heterodimerization complex as reference, have shown that it is possible to trap c-Jun into an inactive complex by using recombinant proteins featuring the leucine zipper region of c-Fos.<sup>7</sup> This strategy was more effective when the leucine zipper region of c-Fos is modified with an acidic tail designed to increase the affinity by introducing stabilizing electrostatic interactions with the (positively charged) basic region of c-Jun.<sup>8</sup> Therefore, the specificity of these systems relies exclusively on the leucine zipper component of the sequestering agent, which is complementary to that of c-Jun.

We have previously reported oligonucleotide-peptide conjugates that are also capable of interacting with the oncoprotein c-Jun.<sup>9</sup> These systems presented a bivalent surface for the specific simultaneous recognition of both the leucine rich, and the basic region of c-Jun. Unfortunately, although these designs were able to trap the bZIP region of Jun with good affinity at 4 °C, the interaction was relatively weak and independent of the oligonucleotide sequence. Therefore, the conjugates worked as the previously described dominant negative peptides, in which

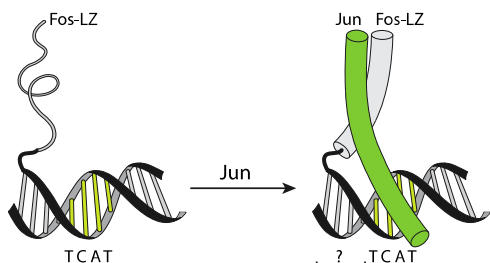
the oligonucleotide unit is just providing a negatively charged surface to favour contacts with the basic region of c-Jun. Moreover, these oligonucleotide-peptide conjugates were not able to disrupt preformed AP-1/DNA complexes.

We have now redesigned the system, and are pleased to report the discovery of a hybrid that binds the bZIP domain of c-Jun at room temperature with high affinity. The interaction of c-Jun with the designed conjugate involves specific contacts of both the leucine zipper region and the oligonucleotidic unit. Importantly, the optimized system allows disassembling of a preformed AP-1 complex.

## Results and Discussion

### Design an optimization of the dsDNA-peptide conjugates

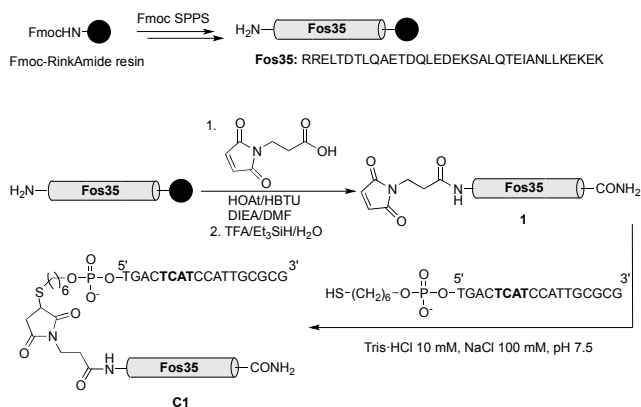
Our previous constructs consisted of a 35 amino acid sequence of c-Fos (residues 158-192) connected through the N terminus to a 5'-thiol terminated oligonucleotide fragment, and featuring a four-base separation between the 5' end and the consensus binding sequence of c-Jun (TCAT). As commented before, gel shift experiments demonstrated that the interaction of the conjugates with c-Jun at 4 °C was independent of the dsDNA sequence,<sup>9</sup> suggesting that the basic region of c-Jun interacts with the polyphosphate surface in a nonspecific manner, instead of being inserted in the DNA groove forming specific contacts with the edges of the bases, as happens in the natural AP-1 complex. A qualitative model based on the X-ray structure of the DNA/AP-1 complex suggested that the inability to form specific contacts could arise from an inappropriate arrangement of the c-Jun DNA binding site, which would not allow a strain-less insertion of the c-Jun basic region into the DNA major groove. We envisioned that having a five or even six-base pair spacer between the consensus binding sequence of c-Jun and the 5' end of the DNA might increase the flexibility of the system, thereby allowing the insertion of the basic region into the groove (Fig. 1). On the other hand, since our previous double stranded oligonucleotide-peptide conjugates also underwent partial dehybridization in the binding assays, we decided to use slightly longer oligonucleotide chains to increase the thermal stability.



**Fig. 1** Strategy for the specific recognition of the bZIP domain of the oncoprotein c-Jun using ds-oligonucleotide-peptide conjugates. The question mark indicates the (initially unknown) number of base pairs separating the cognate binding site and the 5'-end of the conjugate.

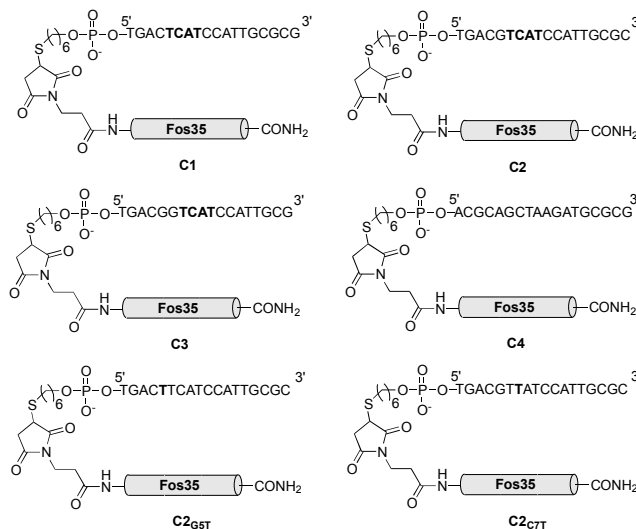
Peptide **1** that contains the leucine-rich region of c-Fos and incorporates a maleimide moiety at the N-terminus, was synthesized on a Rink-4-methyl-benzhydrylamine resin following standard Fmoc/*t*-Bu protocols.<sup>10</sup> The N-terminal maleimide unit was introduced in a final coupling step by using N-maleoyl-β-alanine. Side-chain deprotection and cleavage from the resin (TFA/Et<sub>3</sub>SiH/H<sub>2</sub>O, 95 : 3 : 2) afforded the desired peptide in a reasonable yield (approximately 12%) and with high purity, as deduced from RP-HPLC analysis. Mini-proteins **Fos** and **Jun**, which feature the b-ZIP (basic and leucine rich) regions of the natural proteins c-Fos and c-Jun,<sup>11</sup> were obtained by solid phase synthesis, using a PAL-PEG-PS resin following standard procedures. Side-chain deprotection and cleavage from the resin (TFA/EDT/H<sub>2</sub>O/TIS, 94 : 2.5 : 2.5 : 1) afforded the required peptides.

The coupling reactions between 5'-thiol-terminated oligonucleotides and the maleimide-equipped peptide were carried out by stirring the oligonucleotides (50 μM) with a three-fold excess of the peptide at room temperature (rt), in an aqueous buffer containing 10 mM Tris-HCl and 100 mM NaCl at pH 7.5 (Scheme 1). RP-HPLC monitoring of the reaction revealed the relatively rapid formation of new products that had the chromatographic and spectroscopic characteristics expected for the oligonucleotide-peptide conjugates (λ<sub>max</sub>: 220 and 260 nm, full conversion after 30 min). After HPLC purification, the identity of the products was confirmed by MALDI-MS (see the ESI). The annealing of all conjugates with their corresponding complementary DNA strands was achieved by mixing equimolar amounts of the conjugates with their complementary oligonucleotides in phosphate buffer 10 mM, NaCl 100 mM, pH 7.5. The double stranded derivatives will be named with the suffix **ds**.



**Scheme 1** Reaction sequence for the synthesis of oligonucleotide-peptide conjugates, exemplified for the case of conjugate **C1**. The consensus binding site for c-Jun is shown in bold.

In addition to **C1**, which includes a four-base pair distance between the consensus binding sequence of c-Jun and the 5' end of the DNA, we also prepared **C2** and **C3**, which have respectively one and two more spacing bases. We also synthesized **C4**, which lacks the c-Jun binding sequence, and conjugates with single mutations in the recognition sequence (**C2<sub>G5T</sub>** and **C2<sub>CTT</sub>**) as controls for specificity.



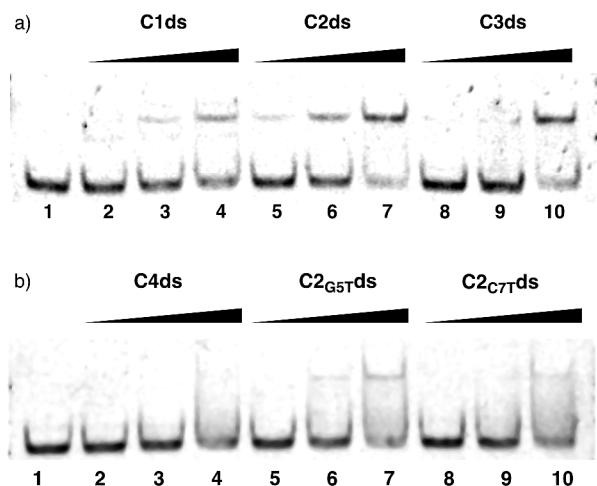
**Scheme 2.** Sequences of the synthesized ss-oligonucleotide-peptide conjugates.

### Interaction assays of Jun with the oligonucleotide-peptide conjugates

With the ds-oligonucleotide-peptide conjugates at hand, we were in position to run preliminary binding assays with **Jun**, which contains the bZIP functional region of the natural protein c-Jun. The interaction was studied by electrophoretic mobility shift assays (EMSA) under non-denaturing conditions.<sup>12</sup> As shown in the gel-shift results (Fig 2a), we observed the formation of retarded bands when **Jun** was added to the double-stranded conjugates **C1ds**, **C2ds**, **C3ds**. Interestingly, we observed qualitative differences in the binding properties of the three conjugates, with the ones featuring a five or a six base pair spacer between the edge of the DNA and the target sequence showing a better affinity. In particular, **C2ds**, which contains a 5-base pair spacing, is the one presenting the best properties in terms of affinity. This result confirmed our initial hypothesis that the spacer between the 5' end of the DNA and the consensus-binding site of c-Jun has an important influence in the formation of the complex.

We next ran several control binding assays in order to study the specificity of the interaction. Importantly, a conjugate that features a dsDNA in which the consensus c-Jun binding sequence has been fully mutated (**C4ds**) failed to elicit retarded bands when treated with the same concentrations of **Jun** than in the experiments above (Fig 2b, lanes 2-4). Moreover, even the conjugates in which the oligonucleotide sequence exhibits a single mutation in the consensus recognition site (**C2<sub>G5T</sub>ds** and **C2<sub>CTT</sub>ds**) were also unable to show significant **Jun** trapping

ability (Fig 2b, lanes 5-10).



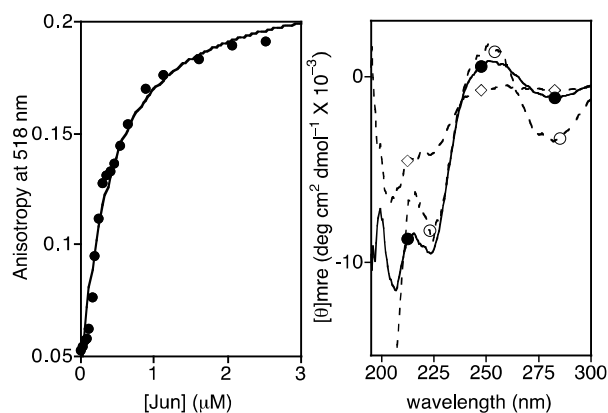
**Fig. 2** EMSA analysis of **Jun** binding to ds-oligonucleotide-peptide conjugates at 22 °C (SyBR gold staining). a) Lane 1: **C1ds** 50 nM; Lanes 2-4: **C1ds** 50 nM with **Jun** 250, 500 and 1000 nM; Lanes 5-7: **C2ds** 50 nM with **Jun** 250, 500 and 1000 nM; Lanes 8-10: **C3ds** 50 nM with **Jun** 250, 500 and 1000 nM. b) Lane 1: **C4ds** 50 nM; Lanes 2-4: **C4ds** 50 nM with **Jun** 250, 500 and 1000 nM; Lanes 5-7: **C2<sub>G5T</sub>ds** 50 nM with **Jun** 250, 500 and 1000 nM; Lanes 8-10: **C2<sub>C7T</sub>ds** 50 nM with **Jun** 250, 500 and 1000 nM. For details in the conditions of the assays, see Experimental Section.

A titration with **Jun** using **C2ds** containing a  $^{32}\text{P}$  radioactively labelled strand, revealed a dissociation constant of  $\approx 201$  nM at room temperature (see Fig. S1 and S2 in the ESI). To obtain a more quantitative characterization of the recognition process, we also performed fluorescence anisotropy titrations. Thus, incubation of a fluorescein-labelled **C2ds** with increasing amounts of **Jun** resulted in a progressive increase of the anisotropy, which was consistent with the formation of a complex with higher molecular weight and reduced mobility.<sup>13</sup> Fitting the resulting data to a 1:1 binding model allowed to calculate its dissociation constant ( $K_d \approx 362$  nM) at 22°C.

A qualitative assessment of structural changes associated to the formation of the complex between the conjugate **C2ds** and **Jun** was obtained by circular dichroism. Therefore, while **C2ds** and the protein **Jun** are poorly structured as isolated entities (Fig. 3 right, dashed lines), mixing them in equimolar amounts produces an increase in the negative ellipticity of the bands at 208 and 222 nm that correlates with an increase of  $\alpha$ -helical content of these species, as expected for the formation of the complex. Moreover, the CD spectrum also indicates a small increase in the intensity of the negative peak at 275 nm upon addition of **Jun**, which suggests that there is some degree of unwinding in the DNA structure.<sup>14</sup>

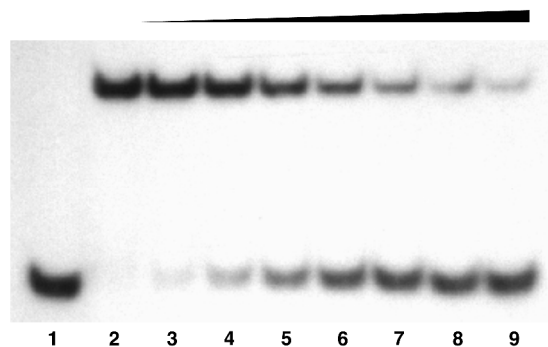
With the conjugate **C2ds** at hand, we were in position to assess whether it could interfere with or even destroy the AP-1/DNA complex. Toward this end, a  $^{32}\text{P}$ -labelled double stranded (ds) oligonucleotide containing the AP-1 binding site was incubated with equimolar amounts of **Fos** and **Jun** to give the expected complex as deduced from the formation of a retarded EMSA band (Fig 4, lane 2).<sup>15</sup> Gratifyingly, incubation of this mixture with increasing amounts of the ds conjugate **C2ds** promoted the progressive disappearance of the slower migrating band. A titration allowed us to calculate an  $\text{IC}_{50}$  of  $\approx 491$  nM (see Fig. S3

in the ESI).



**Fig. 3** Left: Fluorescence anisotropy titration at 518 nm of a fluorescein-labelled **C2ds** (50 nM) with increasing amounts of **Jun** at 22 °C in 10 mM Tris-HCl (pH 7.5), and best fit to a simple 1:1 binding model. Right: Circular Dichroism of **C2ds** in presence of **Jun** at 22 °C in 10 mM Tris-HCl (pH 7.5), 100 mM NaCl. Dashed lines: ( $\diamond$ ) **Jun** (5  $\mu\text{M}$ ); ( $\circ$ ) **C2ds** (5  $\mu\text{M}$ ). Solid line ( $\bullet$ ) **C2ds** (5  $\mu\text{M}$ ) with **Jun** (5  $\mu\text{M}$ ).

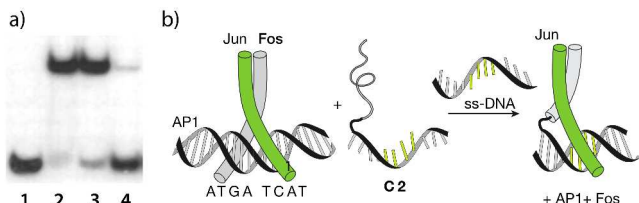
Therefore, this result demonstrates that our ds-oligonucleotide-peptide conjugate **C2ds** is not only capable of binding free **Jun**, but it can also disrupt preformed AP-1-like complexes.



**Fig. 4** EMSA analysis of the competition assay of DNA/**Fos**-**Jun** complex with increasing amounts of conjugate **C2ds** at 22 °C Lane 1-9:  $^{32}\text{P}$ -radiolabelled dsDNA 90 pM; Lane 2: with **Fos** and **Jun** 500 nM; Lane 3-9: same as lane 2 with increasing amounts of conjugate **C2ds**: 150, 250, 400, 550, 800, 1000 and 1250 nM. See the Experimental Section for details.

A control experiment using the conjugate **C2**, which contains a single-stranded oligonucleotide strand, revealed that, as expected, this compound is not able to disassemble the AP-1/DNA complex (Fig. 5., lane 3). However, addition to the previous mixture of a DNA strand complementary to that in conjugate **C2** promoted the disappearance of the band corresponding to the AP-1 complex (Fig 5, lane 4). This experiment, in addition to confirming the requirement of the dsDNA to provide a specific interaction site for the basic region of **Jun**, also hints to an interesting strategy for the conditional activation of the system by using external ssDNAs.





**Fig. 5** EMSA analysis of the disruption of the AP-1 complex at 22 °C. a) Lane 1-4:  $^{32}\text{P}$ -radiolabelled dsDNA 90 pM; Lane 2: with **Fos** and **Jun** 500 nM; Lane 3: same as lane 2 with conjugate **C2** 1500 nM; Lane 4: same as lane 3 with complementary DNA strand to conjugate **C2** 1500 nM. b) Illustration of the conditional sequestration of **Jun** and consequent AP-1 disruption.

## Conclusions

In summary, our data demonstrate that an appropriately designed peptide-oligonucleotide conjugate that present both a DNA and a peptidic recognition surface can bind transcription factors fragments by means of a specific, bivalent interaction, and even disrupt their DNA complexes. This strategy offers unique opportunities to exploring relevant biomolecular recognition processes, as well as to developing new types of conditional decoy platforms for specific sequestration of transcription factors that, as c-Jun, are involved in the progress of prominent diseases like cancers.

## Experimental Section

### Peptide synthesis

All peptide synthesis reagents and amino acid derivatives were purchased from *GL Biochem* (Shanghai) and *NovaBiochem*; standard amino acids were purchased as protected Fmoc amino acids with the standard side chain protecting scheme. C-terminal amide peptides were synthesized using a 0.66 mmol/g loading Rink-MBHA amide resin from *GL Biochem* (Shanghai) or a 0.21 mmol/g loading Fmoc-PAL-PEG-PS resin from *Applied Biosystems*. All other chemicals were purchased from *Sigma-Aldrich*. All solvents were dry and synthesis grade, except DMF for peptide synthesis.

The synthesis of peptide **1** was carried out manually on a Rink-MBHA amide resin (0.66 mmol/g, 0.3 mmol scale) using the following protocol: 1) DMF washings,  $5 \times 1$  min; 2) piperidine/toluene/DMF (2:1:7, 10 mL),  $2 \times 2$  min; 3) DMF washings,  $4 \times 1$  min; 4)  $\text{CH}_2\text{Cl}_2$  washing,  $2 \times 1$  min; 5) Fmoc-amino acid (8 eq, 2.4 mmol) and DIC (4 eq, 1.2 mmol) in DMF /  $\text{CH}_2\text{Cl}_2$  (3 mL/0.6 mL), 1 h. The completion of the coupling reaction was monitored using Kaiser and NF-31 tests. If one of the tests was positive, the coupling reaction was repeated with 4 eq of Fmoc-amino acid, HOAt (4 eq, 1.2 mmol) and HBTU (4 eq, 1.2 mmol) and DIEA (8 eq, 2.4 mmol) in DMF (2 mL). After coupling of the last arginine, an aliquot of resin-bound peptide (50 mg, 0.01 mmol scale) was separated and it was reacted with a solution of N-maleoyl- $\beta$ -alanine (BMPA, 5 eq, 8.5 mg, 0.05 mmol), HOAt (5 eq, 6.8 mg, 0.05 mmol), HBTU (5 eq, 19.0 mg, 0.05 mmol) and DIEA (10 eq, 17  $\mu\text{L}$ , 0.1 mmol) in DMF (600  $\mu\text{L}$ ). The cleavage/deprotection step was performed by treatment of the resin-bound peptide with the following mixture: 950  $\mu\text{L}$  of TFA, 30  $\mu\text{L}$  of  $\text{Et}_3\text{Si}$  and 20  $\mu\text{L}$  of  $\text{H}_2\text{O}$  (300  $\mu\text{L}$  of this mixture for 10 mg of resin). After precipitation in  $\text{Et}_2\text{O}$ , the peptide was

dissolved in  $\text{H}_2\text{O}$  / 0.1% TFA (500  $\mu\text{L}$ ). Reversed-phase HPLC analysis (performed using an *Agilent 1100* series Liquid Chromatograph Mass Spectrometer system, *Zorbax Eclipse XDB-C18* (5  $\mu\text{m}$ ) 4.6  $\times$  150 mm analytical column from *Agilent*, gradient of 5 to 95%  $\text{CH}_3\text{CN}$ , 0.1% TFA /  $\text{H}_2\text{O}$ , 0.1% TFA over 30 min, detection at 220 nm, Electrospray Ionization Mass Spectrometry (ESI/MS) (performed with an *Agilent 1100* Series LC/MSD *VL G1956A* model in positive scan mode) revealed that conjugate **1** elutes with a retention time of 13.5 min. The purification of the peptide was performed on a *Jupiter Proteo 90A* (4  $\mu\text{m}$ ) 10  $\times$  250 mm reverse-phase column from *Phenomenex* and the peptide was identified by ESI/MS ( $[\text{MH}]^+$  calculated for  $\text{C}_{179}\text{H}_{300}\text{N}_{51}\text{O}_{67}$  4238.7, found 1413.4  $[\text{MH}_3]^{3+}$ ; 1060.5  $[\text{MH}_4]^{4+}$ ; 848.3  $[\text{MH}_5]^{5+}$ ). Approximate yield for the whole process 12%.

The miniprotein **Fos** was synthesized using an automatic peptide synthesizer from *Protein Technologies PS3 Peptide Synthesizer* on a Fmoc-PAL-PEG-PS resin (0.21 mmol/g, 0.05 mmol scale). The amino acids were coupled in 4-fold excess using HBTU as activating agent. Each amino acid was activated for 30 seconds in DMF before being added onto the resin. Peptide bond-forming couplings were conducted for 30 min to 45min. The deprotection of the temporal Fmoc protecting group was performed by treating the resin with 20% piperidine in DMF solution for 10 min. The cleavage/deprotection step was performed by treatment of the resin-bound peptide (0.02 mmol) with 3 mL of the cleavage cocktail (75  $\mu\text{L}$  of 1,2-ethanedithiol (EDT), 75  $\mu\text{L}$  of water, 30  $\mu\text{L}$  of triisopropylsilane (TIS) and TFA to 3 mL) for 3 h. After precipitation in  $\text{Et}_2\text{O}$ , the peptide was redissolved in acetonitrile/water 1:1 (1 mL). Reversed-phase HPLC analysis (same conditions as peptide **1**, gradient of 15 to 95%  $\text{CH}_3\text{CN}$ , 0.1% TFA /  $\text{H}_2\text{O}$ , 0.1% TFA over 30 min) revealed that **Fos** elutes with a retention time of 15.5 min. The purification of the peptide was performed on a *Jupiter Proteo 90A* (4  $\mu\text{m}$ ) 10  $\times$  250 mm reverse-phase column from *Phenomenex* and the peptide was identified by ESI/MS ( $[\text{MH}]^+$  calculated for  $\text{C}_{290}\text{H}_{503}\text{N}_{99}\text{O}_{90}\text{S}_3$  6909.70, found 1383.4  $[\text{MH}_3]^{5+}$ ; 1152.9  $[\text{MH}_6]^{6+}$ ; 864.9  $[\text{MH}_8]^{8+}$ ). 24% yield for 0.02 mmol scale.

**Jun** was purchased from *Eurogentec* (Belgium) attached to a solid support. The final Fmoc deprotection step was carried out using standard conditions. Reversed-phase HPLC analysis (same conditions as **Fos**) revealed that **Jun** peptide elutes with a retention time of 16.6 min. The purification was performed as previous described, and the peptide was identified by ESI/MS ( $[\text{MH}]^+$  calculated for  $\text{C}_{325}\text{H}_{575}\text{N}_{112}\text{O}_{91}\text{S}_3$  7599.3, found 1267.8  $[\text{MH}_6]^{6+}$ ; 1087.1  $[\text{MH}_7]^{7+}$ ; 950.9  $[\text{MH}_8]^{8+}$ ; 845.5  $[\text{MH}_9]^{9+}$ ; 760.9  $[\text{MH}_{10}]^{10+}$ ).

### Synthesis of the oligonucleotide-peptide hybrids

The DNA oligos were purchased from *Thermo Fisher Scientific*. The conjugation reactions were performed in aqueous buffer solution (10 mM Tris-HCl, 100 mM NaCl, pH 7.5) with thiol-oligo (50  $\mu\text{M}$ ) and a threefold excess of the peptide **1** (150  $\mu\text{M}$ ). The conjugation reaction was monitored by RP-HPLC (Waters X-Terra column at a flow rate of 1 mL/min, detection at 220 and 260 nm, gradient 0 to 50 % B over 20 min, A: 5%  $\text{CH}_3\text{CN}$  in triethylammonium acetate (TEAA) 100 mM, pH 7.0, B: 70%  $\text{CH}_3\text{CN}$  in TEAA 100 mM, pH 7.0). The oligonucleotide-peptide

conjugates were purified by reversed-phase HPLC and were identified by MS-MALDI-TOF. **C1**:  $t_R = 15.9$  min,  $[\text{MH}]^+$  calculated for  $\text{C}_{359}\text{H}_{535}\text{N}_{114}\text{O}_{178}\text{P}_{18}\text{S}$  9880,12, found 9880.66; **C2**:  $t_R = 15.8$  min,  $[\text{MH}]^+$  calculated for  $\text{C}_{359}\text{H}_{535}\text{N}_{114}\text{O}_{178}\text{P}_{18}\text{S}$  9880,12, found 9879.60; **C3**:  $t_R = 15.9$  min,  $[\text{MH}]^+$  calculated for  $\text{C}_{360}\text{H}_{535}\text{N}_{116}\text{O}_{178}\text{P}_{18}\text{S}$  9920,13, found 9921.50; **C4**:  $t_R = 15.4$  min,  $[\text{MH}]^+$  calculated for  $\text{C}_{360}\text{H}_{532}\text{N}_{125}\text{O}_{173}\text{P}_{18}\text{S}$  9963,16, found 9964.57; **C2<sub>G5T</sub>**:  $t_R = 15.5$  min,  $[\text{MH}]^+$  calculated for  $\text{C}_{359}\text{H}_{536}\text{N}_{111}\text{O}_{179}\text{P}_{18}\text{S}$  9855,12, found 9854.97; **C2<sub>C7T</sub>**:  $t_R = 15.6$  min,  $[\text{MH}]^+$  calculated for  $\text{C}_{360}\text{H}_{536}\text{N}_{113}\text{O}_{179}\text{P}_{18}\text{S}$  9895,12, found 9896.05.

#### Annealing of the conjugates with complementary oligonucleotides

The oligonucleotide-peptide conjugates were annealed with their complementary DNA strands by mixing equimolecular amounts of each conjugate and its complementary oligonucleotide in phosphate buffer 10 mM, NaCl 100 mM, pH 7.5. The solutions were heated to 90 °C for 10 min and then allowed to slowly cool to room temperature.

#### Binding assays

For gel mobility-shift assays, binding reactions with miniprotein **Jun** were performed over 30 min, at 22 °C by using ds-oligonucleotide-peptide conjugates ( $\approx 50$  nM) in a binding mixture containing Tris-HCl (18 mM, pH 7.5), KCl (90 mM), MgCl<sub>2</sub> (1.8 mM), EDTA (1.8 mM), 9 % glycerol, bovine serum albumin (0.11 mg/mL) and 2.2 % NP-40. Products were resolved by using a 15 % nondenaturing polyacrylamide gel and 0.5× TBE buffer (140 V, 60 min, 22 °C) and stained with SYBR® Gold Nucleic Acid Gel Stain (*Molecular Probes*) according to the standard protocol.

The titration of **Jun** was carried out with  $\approx 90$  pM labelled ds-oligonucleotide-peptide conjugate, constructed by annealing **C2** with a <sup>32</sup>P-labelled complementary ss-DNA. The competition assays of **Fos-Jun** complex with conjugate **C2ds** was carried out with  $\approx 90$  pM <sup>32</sup>P-labelled ds-DNA, **Jun**  $\approx 500$  nM and **Fos**  $\approx 500$  nM with increasing amounts of conjugate **C2ds**. The experiments were carried out in the standard binding buffer at 22 °C. The <sup>32</sup>P-labelling was carried out using standard kinase methods. Products were resolved by PAGE using a 15% nondenaturing polyacrylamide gel and 0.5× TBE buffer, and analysed by autoradiography.

#### Fluorescence anisotropy

Steady-state fluorescence anisotropy measurements were made in a *Jobin-Yvon Fluoromax-3*, (*DataMax 2.20*), coupled to a temperature controller *Wavelength Electronics LFI-3751*, and using a standard *Hellma* semi-micro cuvette (114F-QS, 10 mm light path). Titrations were carried out at 22 °C using the following parameters:  $\lambda_{\text{ex}} = 495$  nm,  $\lambda_{\text{em}} = 518$  nm, excitation slit width 3.0 nm, emission slit width 12.0 nm, 2 sec integration time, each measure was taken in triplicate. Titrations were made by adding increasing amounts of **Jun** to a solution containing 50 nM labelled ds-oligonucleotide-peptide conjugate, constructed by annealing **C2** with a fluorescein-labelled complementary ssDNA, in 10 mM Tris-HCl, pH 7.5.

#### Circular Dichroism

Circular dichroism measurements were made with a *Jasco J-715* coupled to a *Neslab RTE-111* thermostated water bath, using a *Hellma* 100-QS macro cuvette (2 mm light pass). Measurements were made at 22 °C. Samples contained 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 5  $\mu\text{M}$  of **C2ds** ds-oligonucleotide-peptide conjugate and 5  $\mu\text{M}$  of **Jun**. The spectra are the average of 4 scans and were processed using the “smooth” macro implemented in the program *Kaleidagraph* (v 4.1 by *Synergy Software*).

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#### Notes and references

- <sup>a</sup> *Departamento de Química Orgánica and Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CIQUS), Universidade de Santiago de Compostela, C/ Jenaro de la Fuente s/n, Campus Vida, 15782 Santiago de Compostela, Spain. Fax: (+34) 881 815704; Tel: (+34) 881 814405; E-mail: joseluis.mascareñas@usc.es*
- † Electronic Supplementary Information (ESI) available: ESI-MS for peptides **Fos** and **Jun**, and MS-MALDI-TOF for oligonucleotide-peptide conjugates. Titration assay and data fitting for **C2ds** and **Jun**. See DOI: 10.1039/b000000x/
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