Electronic Supplementary Material (ESI) for Chemical Science


# Supporting Information:

# Sensing coiled-coil proteins through conformational modulation of energy transfer processes – selective detection of the oncogenic transcription factor c-Jun

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**General peptide synthesis procedures**

All peptide synthesis reagents and amino acid derivatives were purchased from *GL Biochem* (Shanghai) and *Novabiochem*; amino acids were purchased as protected Fmoc amino acids with the standard side chain protecting scheme: Fmoc-Ala-OH, Fmoc-Val-OH, Fmoc-Leu-OH, Fmoc-Lys(Boc)-OH, Fmoc-Phe-OH, Fmoc-Ser(tBu)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Trp(Boc)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Ile-OH, Fmoc-Thr(tBu)-OH and Fmoc-Asp(OtBu)-OH except for the orthogonally protected Fmoc-Dap(Alloc)-OH which was purchased from *Bachem* (Cat.#: B-2845). Synthetic c-Jun was purchased from *Eurogentec* (Belgium) attached to a solid support. C-terminal amide peptides were synthesized on a 0.05 mmol scale using a 0.21 mmol/g loading Fmoc-PAL-PEG-PS resin from *Applied Biosystems*. All other chemicals were purchased from *Sigma, Aldrich* or *Fluka*. All solvents were dry and synthesis grade, unless specifically noted.

Peptides were synthesized using an automatic peptide synthesizer from *Protein Tecnologies PS3 Peptide Synthesizer*. The amino acids were coupled in 4-fold excess using *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-N-methylmethanaminium hexafluorophosphate (HBTU) as activating agent. 2-(4,7,10-tris(2-(tert-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl) acetic acid (DOTA($tBu)_3$) was coupled manually in 4-fold excess using 2-(1*H*-7-aza-benzotriazol-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate methanaminium (HATU) 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 final peptides were cleaved from the resin, and side-chain protecting groups were simultaneously removed using a standard TFA cleavage cocktail as outlined below.

High-Performance Liquid Chromatography (HPLC) was performed using an *Agilent 1100* series Liquid Chromatograph Mass Spectrometer system. Analytical HPLC was run using a *Zorbax Eclipse XDB*-$C_8$ (5 µm) 4.6 × 150 mm analytical column from Agilent. The purification of the peptides was performed on a semipreparative *Jupiter Proteo 90A* (4 µm), 10 × 250 mm reverse-phase column from *Phenomenex*. The standard gradient used for analytical and preparative HPLC was 95:15 to 5:95 over 30 min (water/acetonitrile, 0.1% TFA). Electrospray Ionization Mass Spectrometry (ESI/MS) was performed with an *Agilent 1100* Series LC/MSD VL G1956A model in positive scan mode using direct injection of the purified peptide solution into the MS.

UV measurements were made in a *Varian Cary 100 Bio* spectrophotometer using a standard *Hellma* semi-micro cuvette (140.002-QS). Concentrations were measured using the listed extinction coefficients: 195 $M^{-1}$ $cm^{-1}$ at 257 nm for Phe and 5579 $M^{-1}$ $cm^{-1}$ at 278 nm for Trp. [1]

Steady-state emission measurements were made with a *Jobin-Yvon Fluoromax-3*, (DataMax 2.20), coupled to a *Wavelength Electronics* LFI−3751 temperature controller. All measurements were made with a *Hellma* semi-micro cuvette (114F-QS) at 20 ºC, using the following settings: excitation wavelength 280 nm; excitation slit width 4.0 nm, emission slit width 5.0 nm; increment 1.0 nm; integration time 0.20 s. The emission spectra were recorded from 450 to 600 nm with a 370 nm long-pass filter to avoid interference from harmonic doubling.

Time-gated emission measurements were made with a *Varian Cary Eclipse* Fluorescence Spectrophotometer. All measurements were made with a *Hellma* semi-micro cuvette (114F-QS) at 20 ºC, using the following settings: excitation wavelength 280 nm; excitation slit width 20.0 nm, emission slit width 5.0 nm; increment 1.0 nm; average time 0.05 s; total decay time 0.02 s; delay time 0.2 ms; PMT detector voltage 800 v.

Circular dichroism measurements were made with a *Jasco J-715* coupled to a *Neslab RTE-111* termostated water bath, using a *Hellma* 100-QS macro cuvette (2 mm light pass). Measurements were made at 20 ºC. Samples contained 10 mM HEPES buffer (pH 7.6), 100 mM NaCl, 5 μM of DOTA[Tb]-peptides and 5 μM of partner peptides when present. The spectra are the average of 4 scans and were processed using the "smooth" macro implemented in the program *Kaleidagraph* (v 3.5 by *Synergy* Software).

[1] Fasman, G.D. *Handbook of Biochemistry and Molecular Biology, Proteins, I* **1976**, CRC Press, 3 ed., pp. 183–203.

## Outline of the peptide synthesis strategies

The sequences of the different peptides are:

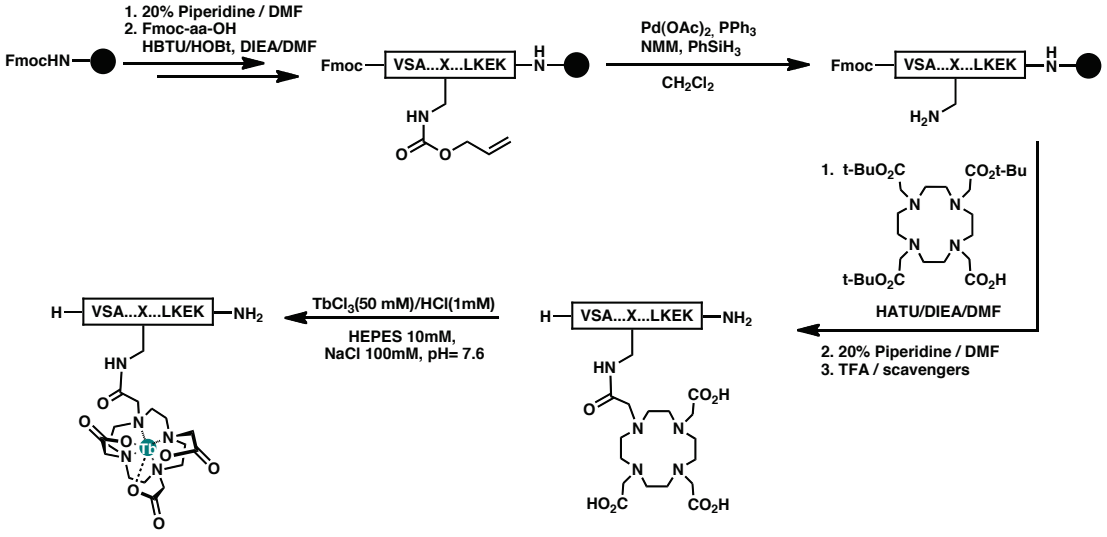
```
                    abcdefg abcdefg abcdefg abcdefg
E4      -  FVSALEKE VSALEKE VSALEKE VSALEKE
K4      -   VSALKEK VSALKEK VSALKEK VSALKEK
W9K4    -   VSALKEK VWALKϕK VSALKEK VSALKEK
W17K4   -   VSALKEK VSALKϕK VSWLKEK VSALKEK
W9/17K4 -   VSALKEK VWALKϕK VSWLKEK VSALKEK
                    9       13      17
```

**Scheme S1**. Sequences of **$E_4$/$K_4$** peptides.

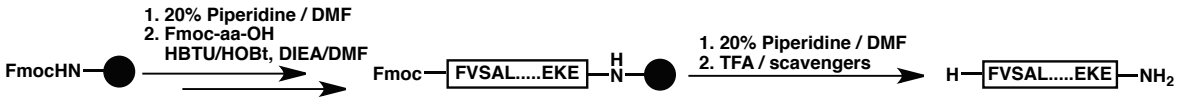
```
                                                        H1      H2      H3      H4      H5
                                                    abcdefg abcdefg abcdefg abcdefg abcdefg abcd
Fos[W][Tb]      -                          LAELQAE TDQLEϕE KSWLQTE IANLLKE KEKL
A-Fos[W][Tb]    -              EELEKEAEELEQEL LAELQAE TDQLEϕE KSWLQTE IANLLKE KEKL
c-Fos           -   MKRRIRRERNKMAAAKCRNRRREL TDTLQAE TDQLEDE KSALQTE IANLLKE KEKLW
c-Jun           -  FIKAERKRMRNRIAASKSRKRKLER IARLEEK VKTLKAQ NSELAST ANMLREQ VAQLKQK VMNH
```

**Scheme S2**. Sequences of **c-Fos/c-Jun** and sensor peptides, where ϕ = Dap(DOTA[$Tb^{3+}$]).

The strategy for the synthesis of side-chain DOTA-conjugated peptides **$^{W9}K_4$**, **$^{W17}K_4$**, **$^{W9/17}K_4$**, **Fos[W][Tb]** and **A-Fos[W][Tb]** involved a modified protecting group scheme of the standard Fmoc solid phase peptide synthesis tactic. The 2,3-diaminopropionic acid (Dap) residue is introduced as an (allyloxy)carbonyl-protected derivative (Alloc-protected), which allowed its selective side chain deprotection (Pd catalysis) and derivatization with DOTA.




**Scheme S3**. Synthesis of Tb-peptide **$^{W17}K_4$** complex whit the DOTA unit orthogonally attached to the Dap side chain. The same synthetic scheme was followed for the synthesis of **$^{W9}K_4$**, **$^{W9/17}K_4$**, **Fos[W][Tb]** and **A-Fos[W][Tb]**.




**Scheme S4.** Synthesis of peptide $\mathbf{E_4}$.

**Deprotection of the orthogonally protected Dap(Alloc) side chain:** Once the peptides were fully assembled in the solid phase, the side chain of the Dap(Alloc) residue is selectively deprotected for specific attachment of the DOTA unit following in all cases the same procedure: 0.05 mmol of peptide attached to the solid support were treated at room temperature for 12 h with a mixture of $Pd(OAc)_2$ (0.3 eq), $PPh_3$ (1.5 eq), N-Methylmorpholine (NMM) (10 eq), and $PhSiH_3$ (10eq) in $CH_2Cl_2$ (2.5 mL). The resin was then filtered and washed with THF (1 × 5 mL × 2 min), DMF (2 × 5 mL × 2 min), diethyldithiocarbamate (DEDTC) (25 mg in 5 mL of DMF, 2 × 5 min), DMF (2 × 5 mL × 2 min) and $CH_2Cl_2$ (2 × 5 mL × 2 min).

**Cleavage and deprotection of semipermanent protecting groups:** The resin-bound peptide dried under argon (0.02 mmol) was placed in a 50 mL falcon tube, to which 3 mL of the cleavage cocktail (150 µL of $CH_2Cl_2$, 75 µL of water, 75 µL of triisopropylsilane (TIS) and TFA to 3 mL) were added, and the resulting mixture was shaken for 3 h. The resin was filtered, and the TFA filtrate was concentrated under argon current to a volume of approximately 2 mL. The residue was added to ice-cold diethyl ether (40 mL). After 10 min, the precipitate was centrifuged and washed again with 20 mL of ice-cold ether. The solid residue was dried under argon and redissolved in acetonitrile/water 1:1 (2 mL) and purified by semipreparative reverse-phase HPLC. The collected fractions were lyophilized and stored at −20 °C.

**Peptides $^{W9}K_4$, $^{W17}K_4$, $^{W9/17}K_4$, $Fos^W$[Tb] and A-$Fos^W$[Tb]. DOTA coupling to Dap side chain:** DOTA($t$Bu)$_3$ (35 mg, 0.0608 mmol) was dissolved in dry DMF (800 µL). HATU (23 mg, 0.0608 mmol) and *N,N*-diisopropylethylamine (DIEA) (15.6 µL , 0.0913 mmol) were added to the solution. After two minutes, the resulting mixture was added over the Alloc-deprotected peptide attached to the resin (0.0152 mmol). $N_2$ was passed through the resin suspension for 4 h. The resin was filtered, washed with DMF (3 × 3 mL × 3 min) and subjected to the final Fmoc deprotection step using standard conditions (20% piperidine/DMF). HPLC purification of the crude yielded a white solid identified as the desired product. **Terbium chelation**: The lyophilized peptide was dissolved in 600 µL HEPES buffer 10 mM, pH 7.6, NaCl 100 mM and 400 µL of $TbCl_3$ 50 mM solution in HCl 1 mM was added. The mixture was shaken for 1 h and then HPLC-purified. After lyophilization, we obtained white solids identified as the desired terbium-peptides complexes.

$^{W9}$**K$_4$** : ESI-MS [MH$^+$] calc for $C_{158}H_{275}N_{43}O_{44}Tb$ = 3637.98, found = 3638.2. UV ($H_20$) $\lambda_{max}$, nm (ε): 278 (5579) $M^{-1}cm^{-1}$ (13 mg, 23% yield for 0.015 mmol scale).

$^{W17}$**K$_4$** : ESI-MS [MH$^+$] calc for $C_{158}H_{275}N_{43}O_{45}Tb$ = 3653.97, found = 3656.5. UV ($H_20$) $\lambda_{max}$, nm (ε): 278 (5579) $M^{-1}cm^{-1}$ (10 mg, 18% yield for 0.015 mmol scale).

$^{W9/17}$**K$_4$** : ESI-MS [MH$^+$] calc for $C_{166}H_{280}N_{44}O_{44}Tb$ = 3753.02, found = 3756.2. UV ($H_20$) $\lambda_{max}$, nm (ε): 278 (11158) $M^{-1}cm^{-1}$ (8 mg, 14% yield for 0.015 mmol scale).

**Fos$^W$[Tb]**: ESI-MS [MH$^+$] calc for $C_{182}H_{299}N_{47}O_{63}Tb$ = 4310.08, found = 4311.8. UV ($H_20$) $\lambda_{max}$, nm (ε): 278 (5579) $M^{-1}cm^{-1}$ (12 mg, 19% yield for 0.015 mmol scale).

**A-Fos$^W$[Tb]**: ESI-MS [MH$^+$] calc for $C_{248}H_{402}N_{62}O_{93}Tb$ = 5895.78, found = 5895.8. UV ($H_20$) $\lambda_{max}$, nm (ε): 278 (5579) $M^{-1}cm^{-1}$ (11 mg, 13% yield for 0.015 mmol scale).

**Peptides E$_4$** and **c-Jun:** The final Fmoc deprotection step was carried out using standard conditions and the HPLC purification of the crude yielded white solids identified as the desired products.

**E$_4$** : ESI-MS [MH$^+$] calc for $C_{141}H_{237}N_{34}O_{49}$ = 3190.70, found = 3192.7. UV ($H_20$) $\lambda_{max}$, nm (ε): 257 (195) $M^{-1}cm^{-1}$ (16 mg, 26% yield for 0.02 mmol scale).

**c-Jun** : ESI-MS [MH$^+$] calc for $C_{325}H_{575}N_{112}O_{91}S_3$ = 7599.29, found = 7602.4. UV ($H_20$) $\lambda_{max}$, nm (ε): 257 (195) $M^{-1}cm^{-1}$ (42 mg, 28% yield for 0.02 mmol scale).

**c-Fos** : ESI-MS [MH$^+$] calc for $C_{290}H_{503}N_{99}O_{90}S_3$ = 6909.70, found = 6910.8. UV ($H_20$) $\lambda_{max}$, nm (ε): 278 (5579) $M^{-1}cm^{-1}$ (33 mg, 24% yield for 0.02 mmol scale).

**Preparation of cell lysates**: Asynchronously growing Saos (human osteosarcoma) cells were lysed by sonication in DIP buffer (25 mM HEPES pH 7.5, 75 mM NaCl, 0.5 mM EDTA, 1.25 mM EGTA, 5% glycerol and 0.05% Tween 20, 1 mM NaF, 0.2 mM PMSF and proteinase inhibitor cocktail by *Sigma*). Lysates were clared by centrigufation and protein content in the supernatant was measured by the Bradford method. Protein extracts were stored at −80 ºC until used in the assays.

**Steady-state emission titration of peptide $^{W17}K_4$ with $E_4$.** To 1 mL of a 1 μM solution of peptide $^{W17}K_4$ in HEPES buffer (10 mM HEPES pH 7.6, 100 mM NaCl), aliquots of a 500 μM stock solution of $E_4$ were successively added at 20 °C, and the emission spectra were recorded after each addition.

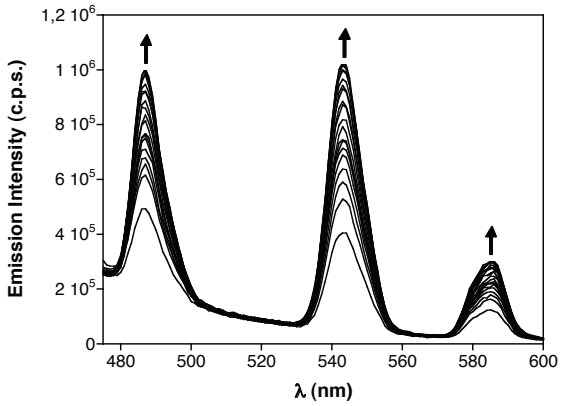



**Figure S1.** Emission spectra of 1 μM peptide $^{W17}K_4$ in presence of peptide $E_4$ in the following concentrations: 0; 0.5; 1; 1.5; 2; 2.5; 3; 3.5; 4; 5; 6; 7; 8.5; 10; 11.5; 13; 15; 17 and 20 μM.

**Time-gated emission titration of peptide $^{W17}K_4$ with $E_4$.** To 1 mL of a 1 μM solution of peptide $^{W17}K_4$ in HEPES buffer (10 mM HEPES pH 7.6, 100 mM NaCl), aliquots of a 300 μM stock solution of $E_4$ were successively added at 20 °C and the emission spectra were recorded after each addition.

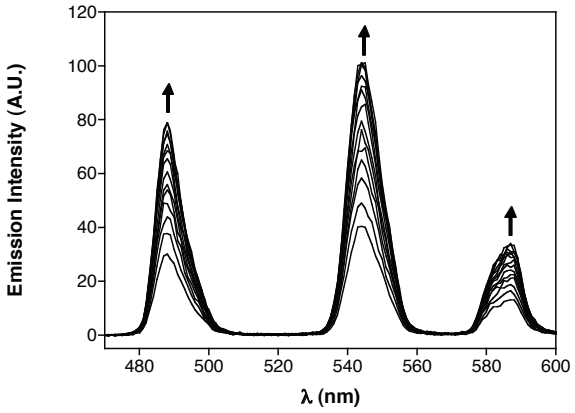



**Figure S2.** Emission spectra of 1 μM peptide $^{W17}K_4$ in presence of peptide $E_4$ in the following concentrations: 0; 1; 2; 3; 4; 5; 6; 8; 10; 12; 14.5; 17 and 20 μM.

**Steady-state emission titration of peptide $^{W9/17}K_4$ with $E_4$.** To 1 mL of a 1 µM solution of peptide $^{W9/17}K_4$ in HEPES buffer (10 mM HEPES pH 7.6, 100 mM NaCl), 1 µL aliquots of a 500 µM stock solution of $E_4$ were successively added at 20 °C and the emission spectra were recorded after each addition.

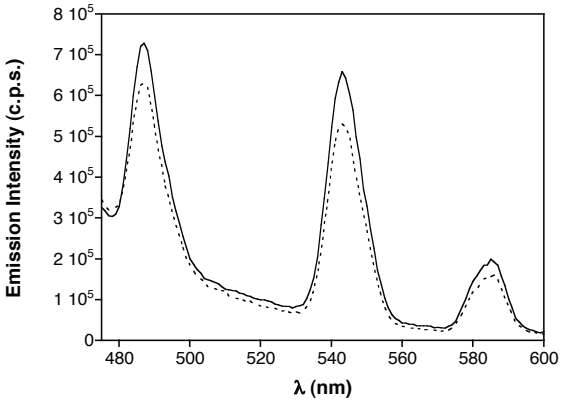



**Figure S3.** Dashed line, emission spectra of 1 µM peptide $^{W9/17}K_4$. Solid line, emission spectra of its complex with $E_4$ (23.8 µM)

**Steady-state emission titration of peptide Fos$^W$[Tb] with c-Jun.** To 1 mL of a 1 µM solution of peptide **Fos$^W$[Tb]** in HEPES buffer (10 mM HEPES pH 7.6, 100 mM NaCl), 4 µL aliquots of a 100 µM stock solution of **c-Jun** were successively added at 20 °C and the emission spectra were recorded after each addition.

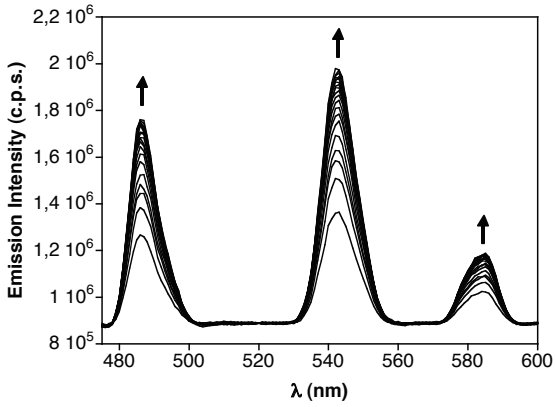



**Figure S4.** Emission spectra of 1 µM peptide **Fos$^W$[Tb]** in presence of peptide **c-Jun** in the following concentrations: 0; 0.4; 0.8; 1.2; 1.6; 2; 2.4; 2.8; 3.2; 3.6; 4; 4.4; 4.8; 5.2; 5.6; 6; 6.4; 6.8; 7.2; 7.6; 8; 8.4; 8.8; and 9.2 µM.

**Steady-state emission titration of peptide A-Fos$^{W}$[Tb] with c-Jun.** To 1 mL of a 1 μM solution of peptide **A-Fos$^{W}$[Tb]** in HEPES buffer (10 mM HEPES pH 7.6, 100 mM NaCl), 4 μL aliquots of a 50 μM stock solution of **c-Jun** were successively added at 20 °C and the emission spectra were recorded after each addition.

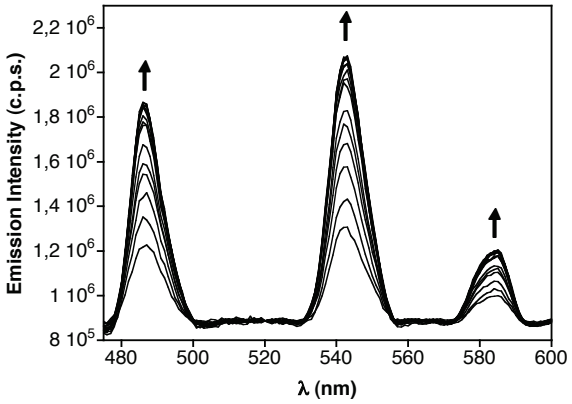



**Figure S5.** Emission spectra of 1 μM peptide **A-Fos$^{W}$[Tb]** in presence of peptide **c-Jun** in the following concentrations: 0; 0.2; 0.4; 0.6; 0.8; 1; 1.2; 1.4; 1.6; 1.8; 2; 2.2; 2.4; 2.6; 2.8; 3; 3.2; 3.4; 3.6 and 3.8 μM.

**Time-gated emission titration of peptide A-Fos$^{W}$[Tb] with c-Jun.** To 1 mL of a 1 μM solution of peptide **A-Fos$^{W}$[Tb]** in HEPES buffer (10 mM HEPES pH 7.6, 100 mM NaCl), 5 μL aliquots of a 100 μM stock solution of **c-Jun** were successively added at 20 °C and the emission spectra were recorded after each addition.

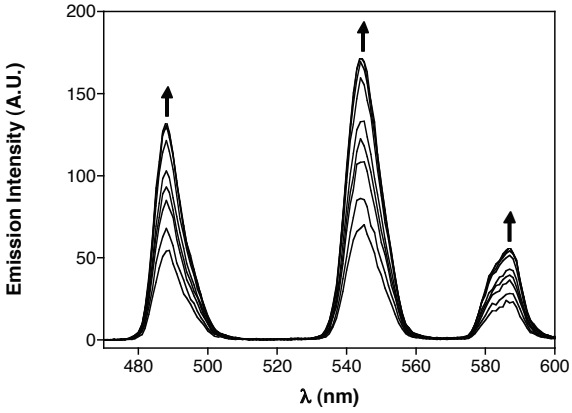



**Figure S6.** Emission spectra of 1 μM peptide **A-Fos$^{W}$[Tb]** in presence of peptide **c-Jun** in the following concentrations: 0; 0.5; 1; 1.5; 2; 2.5; 3; 3.5; and 4 μM.

**Selectivity tests: Steady-state emission of A-Fos$^{W}$[Tb] in the presence of c-Fos and E$_4$.** To 1 mL of a 1 μM solution of peptide **A-Fos$^{W}$[Tb]** in HEPES buffer (10 mM HEPES pH 7.6, 100 mM NaCl), aliquots of **c-Fos** were added up to 8 μM and the emission spectra were recorded after each addition. Experiments with **E$_4$** were performed in the same way at concentration of 10 μM.

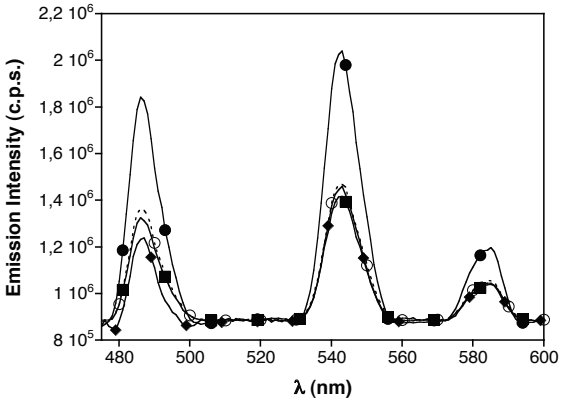



**Figure S7.** Dashed line (○), emission spectra of 1 μM peptide **A-Fos$^{W}$[Tb]**. Solid lines, emission spectra of its complex with 2 equivalents of **c-Jun** (●); control experiment with 8 equivalents of natural **c-Fos** DNA binding domain (◆); control experiment with 10 equivalents of **E$_4$** (■).

**Steady-state emission of A-Fos$^{W}$[Tb]/c-Jun complex treated with c-Fos.** To 1 mL of a 1 μM solution of peptide **A-Fos$^{W}$[Tb]** in HEPES buffer (10 mM HEPES pH 7.6, 100 mM NaCl), 4 equivalents of **c-Jun** were added. Increasing amounts of a 200 μM stock solution of **c-Fos** were added up to 10 μM and the emission spectra were recorded after each addition.

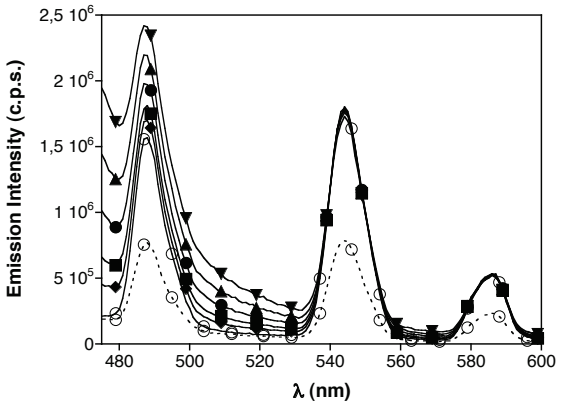



**Figure S8.** Dashed line (○), emission spectra of 1 μM peptide **A-Fos$^{W}$[Tb]**. Solid lines, emission spectra of its complex with 4 equivalents of **c-Jun** (○); and competition assays with 1 equivalent (◆); 2 equivalents (■); 4 equivalents (●); 6 equivalents (▲) and 10 equivalents (▼), of natural **c-Fos** DNA binding domain.

**Steady-state emission of A-Fos$^{W}$[Tb] with cell lysates.** To 1 mL of a 1 µM solution of peptide **A-Fos$^{W}$[Tb]** in HEPES buffer, 7.5 µL of a 8.4 µg/µL stock solution of cell protein extracts were added. 4 µL aliquots of a 50 µM stock solution of **c-Jun** were successively added at 20 °C and the emission spectra were recorded after each addition.

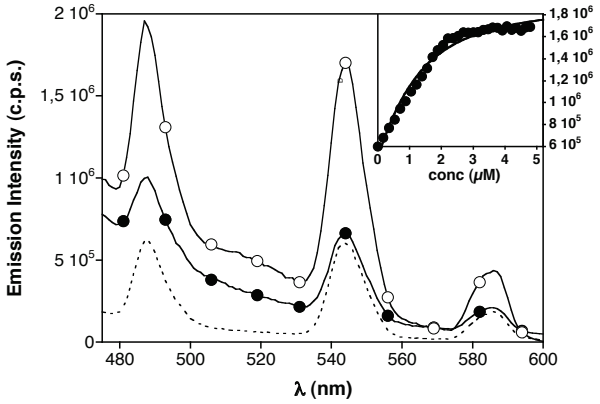



**Figure S9:** Dashed line, emission spectra of 1 µM peptide **A-Fos$^{W}$[Tb]**. Solid lines, emission spectra of peptide **A-Fos$^{W}$[Tb]** with 65 µg/mL of cell protein extracts from cell lysates (●); same sample after addition of 4 equivalents of **c-Jun** (○).Inset with the emission at 545 nm of peptide **A-Fos$^{W}$[Tb]** in the presence of increasing amounts of the complementary **c-Jun**, and the best fitting curve to a 1:1 binding mode.

**Time-gated emission of A-Fos$^{W}$[Tb] with cell lysates.** To 1 mL of a 1 µM solution of peptide **A-Fos$^{W}$[Tb]** in HEPES buffer, 7.5 µL of a 8.4 µg/µL stock solution of cell protein extracts were added. 5 µL aliquots of a 100 µM stock solution of **c-Jun** were successively added at 20 °C and the emission spectra were recorded after each addition.

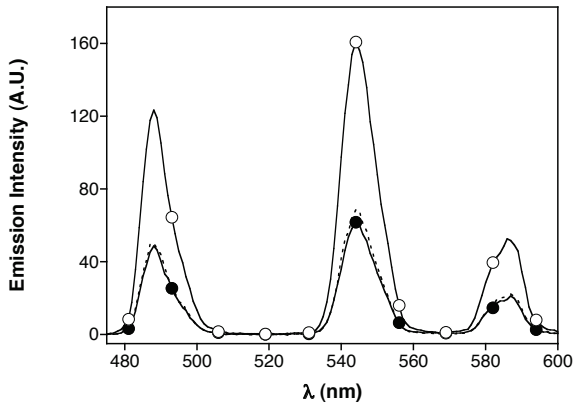



**Figure S10:** Dashed line, emission spectra of 1 µM peptide **A-Fos$^{W}$[Tb]**. Solid lines, emission spectra of peptide **A-Fos$^{W}$[Tb]** with 65 µg/mL of cell protein extracts from cell lysates (●); same sample after addition of 4 equivalents of **c-Jun** (○).

**Circular Dichroism of peptide $^{W17}K_4$ with $E_4$.** To 300 µL of a 5 µM solution of peptide $^{W17}K_4$ in HEPES buffer (10 mM HEPES pH 7.6, 100 mM NaCl), 15 µL of a 100 µM stock solution of $E_4$ were added at 20 °C and the circular dichroism spectra were recorded after the addition.

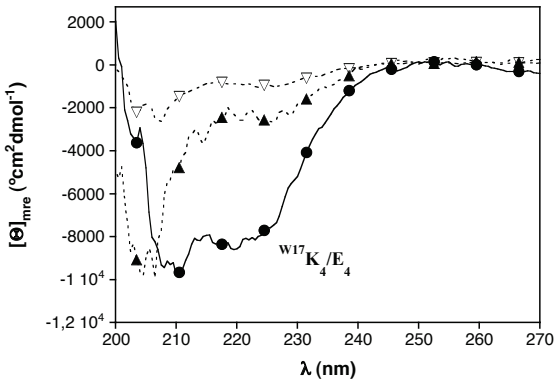



**Figure S11.** Dashed lines, circular dichroism spectra of 5 µM peptide $E_4$ (▽) and 5 µM $^{W17}K_4$ (▲). Solid line, their 1:1 complex (●) at 5 µM concentration.

**Circular Dichroism of peptide A-Fos$^W$[Tb] with c-Jun.** To 300 µL of a 5 µM solution of peptide **A-Fos$^W$[Tb]** in HEPES buffer (10 mM HEPES pH 7.6, 100 mM NaCl), 15 µL of a 100 µM stock solution of **c-Jun** were added at 20 °C and the circular dichroism spectra were recorded after the addition.

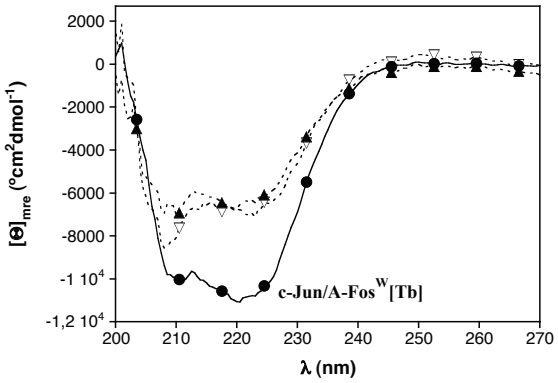



**Figure S12.** Dashed lines, circular dichroism spectra of 5 µM peptide **c-Jun** (▽) and 5 µM **A-Fos$^W$[Tb]** (▲). Solid line, their 1:1 complex (●) at 5 µM concentration.

**Circular Dichroism of peptide A-Fos$^{W}$[Tb] with c-Fos.** To 300 µL of a 5 µM solution of peptide **A-Fos$^{W}$[Tb]** in HEPES buffer (10 mM HEPES pH 7.6, 100 mM NaCl), 15 µL of a 100 µM stock solution of **c-Fos** were added at 20 °C and the circular dichroism spectra were recorded after the addition.

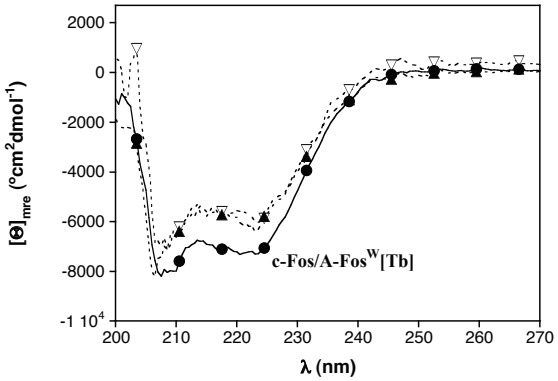



**Figure S13.** Dashed lines, circular dichroism spectra of 5 µM peptide **A-Fos$^{W}$[Tb]** (▽) and 5 µM **c-Fos** (▲). Solid line, their 1:1 complex (●) at 5 µM concentration.

**Data fitting**. The experimental data of the titration of peptide **A-Fos$^{W}$[Tb]** with **c-Jun** were fit to a 1:1 model for a fluorescently-labeled ligand binding (**A-Fos$^{W}$[Tb]**) to a unlabeled receptor (**c-Jun**). If non-specific binding is ignored, then this interaction is described by equations 1−4:

$$K_D = \frac{R \times L}{RL} \quad (1)$$

$$R_T = R + RL \quad (2)$$

$$L_T = L + RL \quad (3)$$

$$F_T = F_0 + F_{RL} \times RL \quad (4)$$

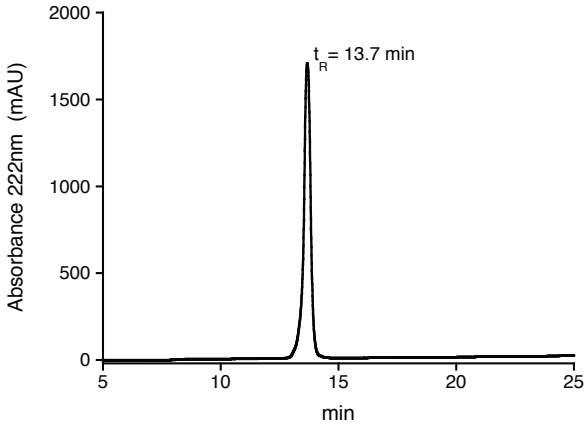
Where $\boldsymbol{R}$, concentration of the free receptor in the equilibrium (**c-Jun**); $\boldsymbol{R_T}$, total receptor concentration; $\boldsymbol{L}$, concentration of the free labeled ligand in the equilibrium (**A-Fos$^{W}$[Tb]**); $\boldsymbol{L_T}$, total concentration of the labeled ligand; $\boldsymbol{K_D}$, dissociation constant of the interaction between the receptor and the ligand; $\boldsymbol{RL}$ concentration of labeled ligand-receptor complex; $\boldsymbol{F_T}$, total observed emission, $\boldsymbol{F_0}$, adjustable parameter accounting for the background emission; $\boldsymbol{F_{RL}}$ adjustable parameter for the labeled ligand-receptor complex molar emission. Solving eqs 1−4 for $\boldsymbol{F_T}$ and eliminating $\boldsymbol{R}$, $\boldsymbol{L}$ and $\boldsymbol{RL}$, we obtain the well-known eq. 5,[2] which was used to fit the experimental data using non-linear regression analysis. Derivation of the equation and data fitting was made with *Mathematica*® 7.0.1.0 program for MacOS X (Wolfram Research).

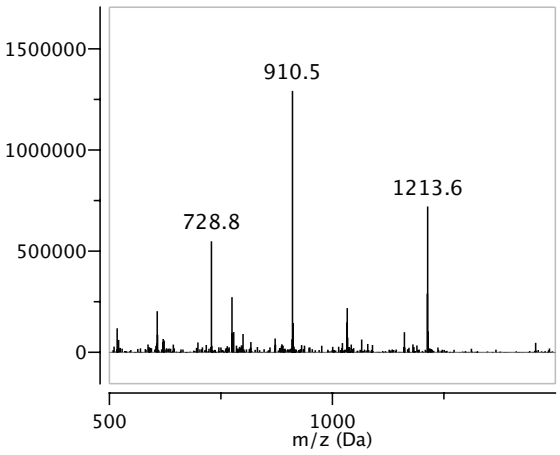
$$F_T = \frac{1}{2}\left(2F_0 + F_{RL} \times \left[K_D + L_T + R_T - \sqrt{\left(K_D + L_T + R_T\right)^2 - 4L_T R_T}\right]\right) \quad (5)$$

[2] Roehrl, M.H.A.; Wang, J.Y.; Wagner, G. *Biochemistry* **2004**, *43*, 16056–16066.

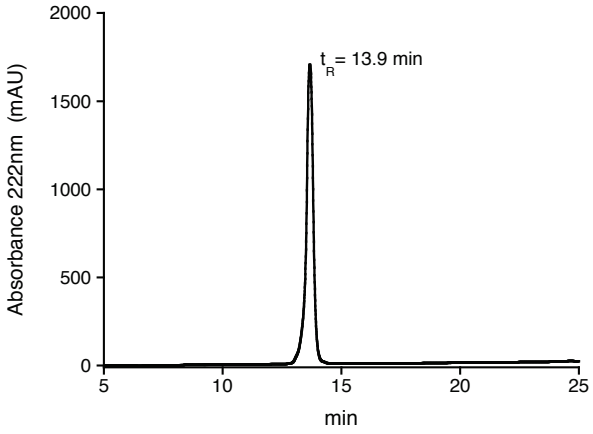
**HPLC and MS-ESI spectra.**

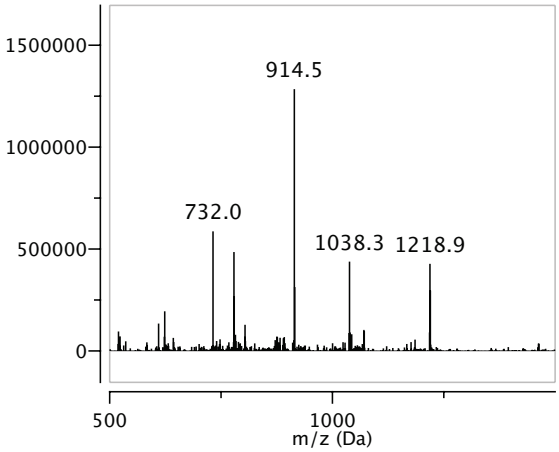
**$^{W9}K_4$.**



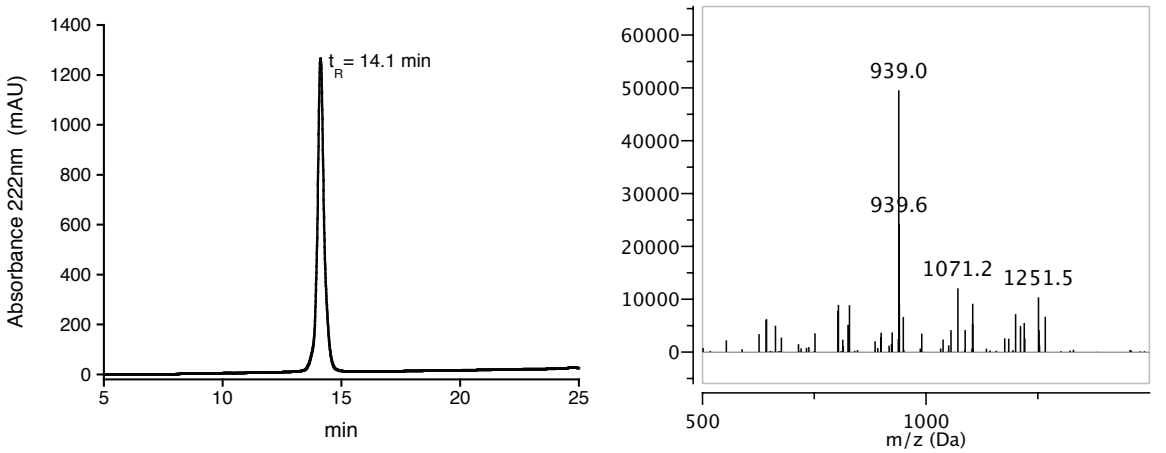




**$^{W17}K_4$.**



$^{W9/17}K_{4.}$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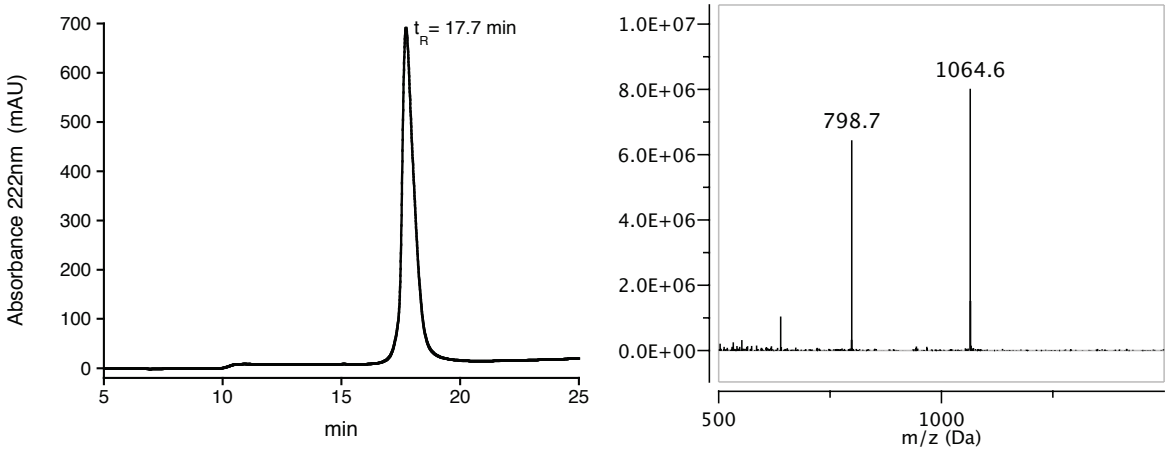
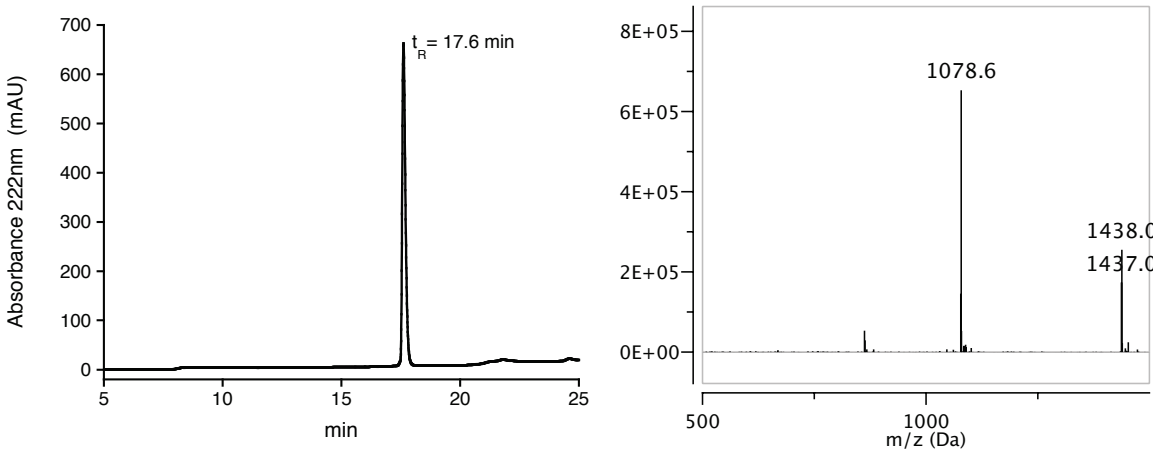
1200
1000
800
600
400
200
0
5
10
15
20
25
min
t_R = 14.1 min
60000
50000
40000
30000
20000
10000
0
939.0
939.6
1071.2
1251.5
500
1000
m/z (Da)

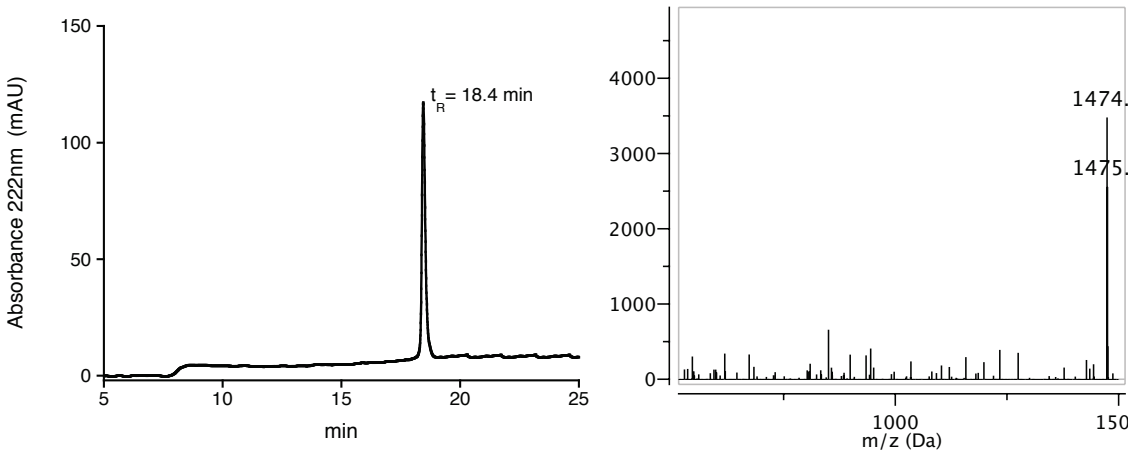
$E_{4.}$


Absorbance 222nm (mAU)
700
600
500
400
300
200
100
0
5
10
15
20
25
min
t_R = 17.7 min
1.0E+07
8.0E+06
6.0E+06
4.0E+06
2.0E+06
0.0E+00
1064.6
798.7
500
1000
m/z (Da)

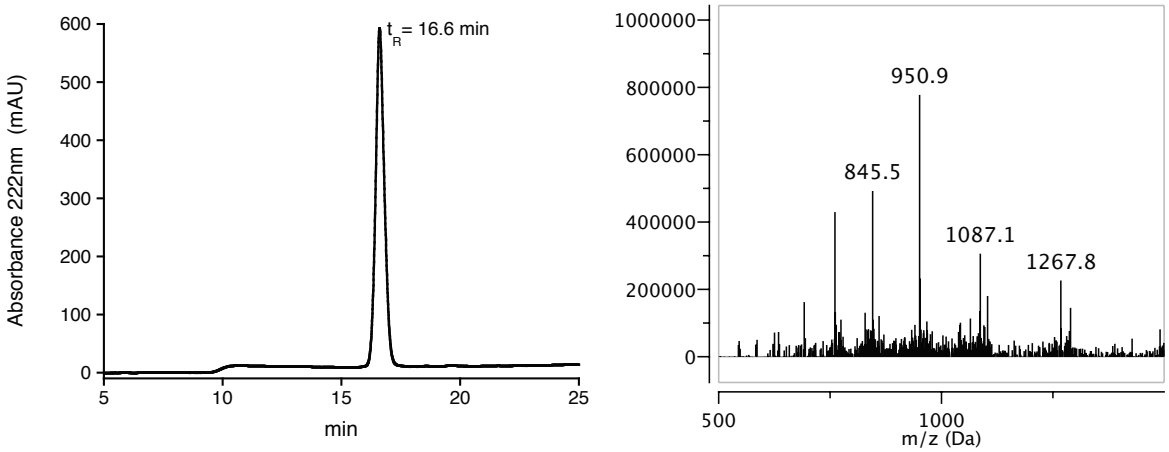
**Fos$^{W}$[Tb].**




**A-Fos$^{W}$[Tb].**



**c-Jun.**




**c-Fos.**