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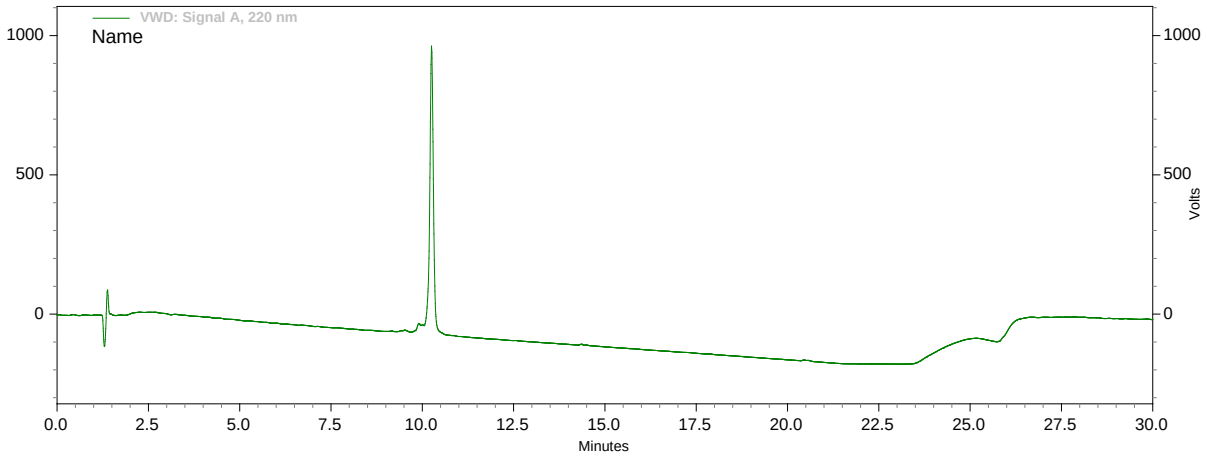
# Clicked bis-PEG-Peptide Conjugates for Studying Calmodulin-Kv7.2 Channel Binding

M. Ángeles Bonache, Alessandro Alaimo, Covadonga Malo, Oscar Millet, Álvaro Villarroel, Rosario González-Muñiz*

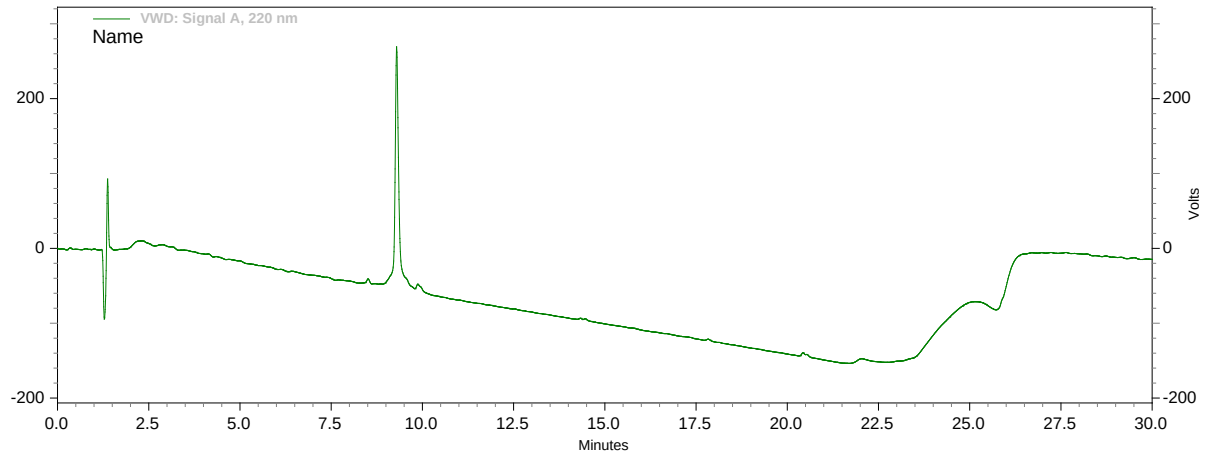
## *Supporting Information*

## 1. General synthetic procedures

Peptides were purified by MPLC using SNAP 12g KP-C18-HS cartridges in an ISOLERA ONE (BIOTAGE). A gradient of $CH_3CN$:$H_2O$ (0.05% TFA) from 0:100 to 30:70 over 60 min as mobile phase, and a flux of 5 mL/min were used. Some peptides were purified by semipreparative RP-HPLC-MS (Waters 2545) coupled to a mass spectrometer 3100 detector, using a SUNFIRE™ column C18 (5µ, 10x150 mm) and a flux of 8 mL/min with a gradient of $CH_3CN$ (0.1% $HCO_2H$) [Solvent A]:$H_2O$ (0.1% $HCO_2H$) [Solvent B] as mobile phase**.** The purity of the peptides was analyzed using an analytical HPLC Waters (model 2690) with a SUNFIRE™ column C18 (3.5 µ, 4.6 x 50 mm) at 1 mL/min with a gradient of $CH_3CN$ (0.005% $HCO_2H$) [Solvent A]:$H_2O$ (0.005% $HCO_2H$) [Solvent B] in 5 min as mobile phase or Agilent Technologies (model 1120 Compact LC ) with Eclipse Plus column C18 (4.6 x150 mm) at 1.5 mL/min with a 5 to 80 gradient of $CH_3CN$ [Solvent A] :$H_2O$ (0.05% TFA) [Solvent B] in 20 min as mobile phase. Characterization of the products was performed by HPLC-MS (Waters) coupled to a single quadrupole ESI-MS (Micromass ZQ 2000) and HRMS (EI+) was carried out in an Agilent 6520 Accurate-Mass Q-TOF LC/MS equipment.

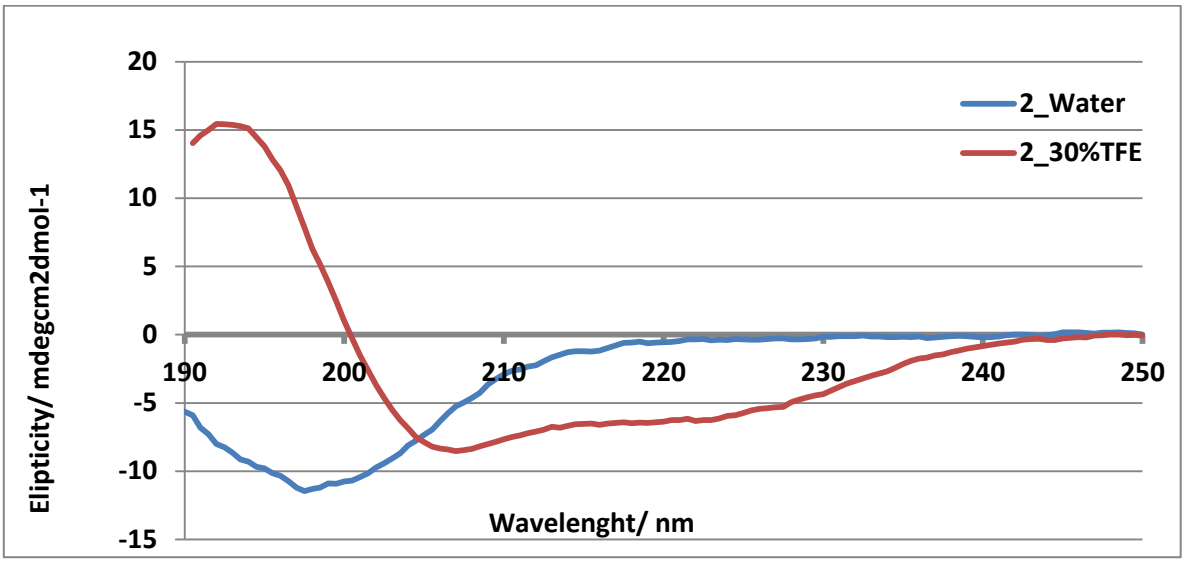


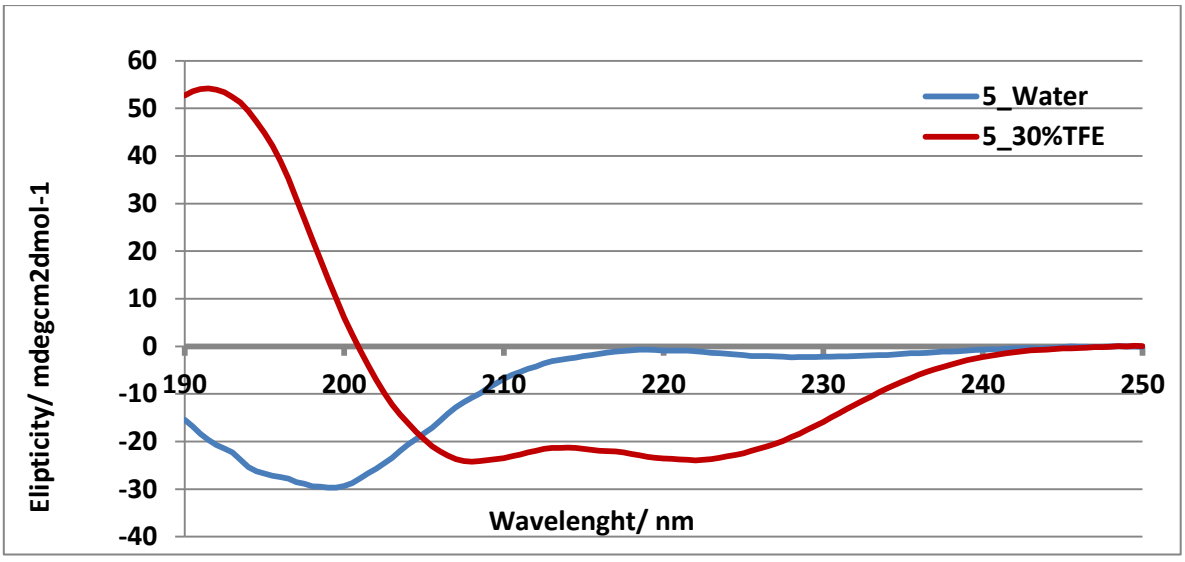

**2**




**5**

**Figure S1**. HPLC chromatograms of purified PEG-peptide conjugated **2** and **5** ($C_{18}$ column, linear gradient from 5 to 80% ACN in 20 min).



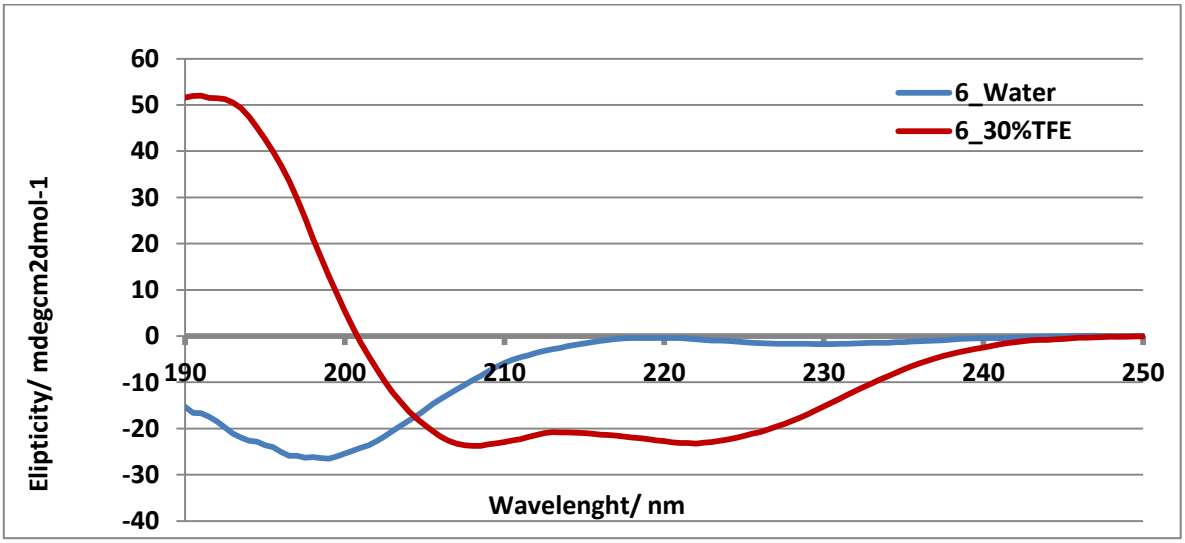




**Figure S2.** CD spectra of peptides **2**, **5** and **6** in $H_2O$ and 30% TFE/$H_2O$ at pH 5.5 and 5 °C.

## 2. CaM binding assays.

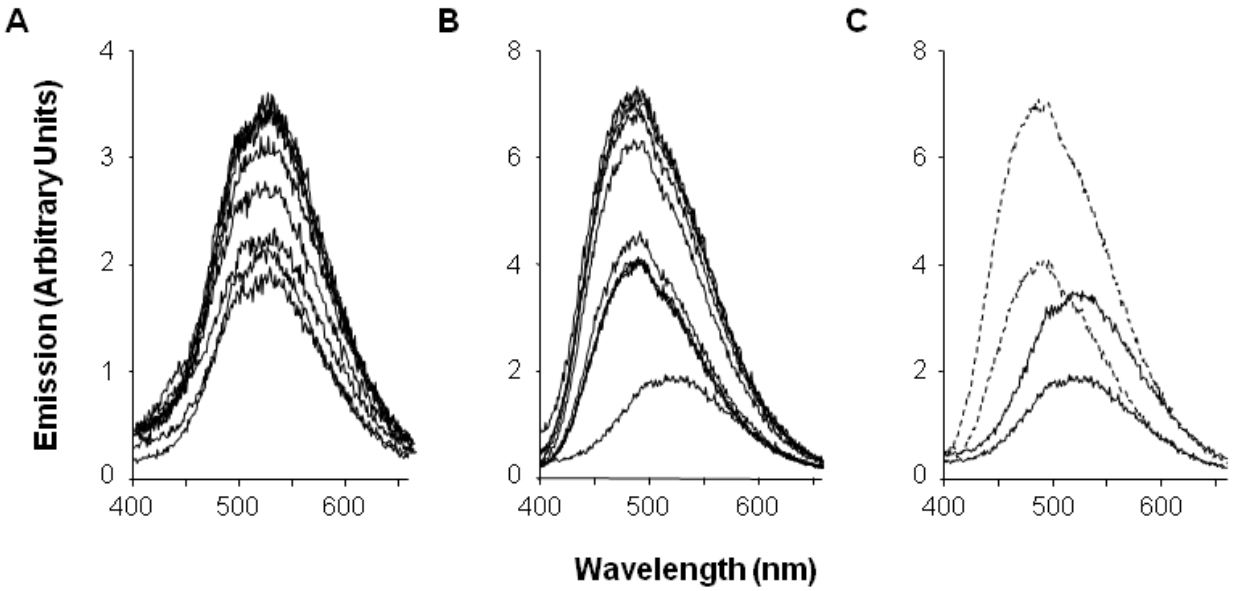



**Figure S3. D-CaM steady-state fluorescence in the presence of peptide 7.** Effect of incremental addition of the peptide conjugate **7** in the emission spectra of 12.5 nM D-CaM in the absence of free $Ca^{2+}$ (**A**, 10 mM EGTA added) and presence of 4 µM free $Ca^{2+}$ (**B**). **C**) Emission spectra of 12.5 nM D-CaM in the presence of 4 µM free $Ca^{2+}$ (dotted lines) and in the absence of $Ca^{2+}$ (solid lines; 10 mM EGTA added), as well as in the presence (bold traces) and absence (light traces) of the **7** (200 nM).

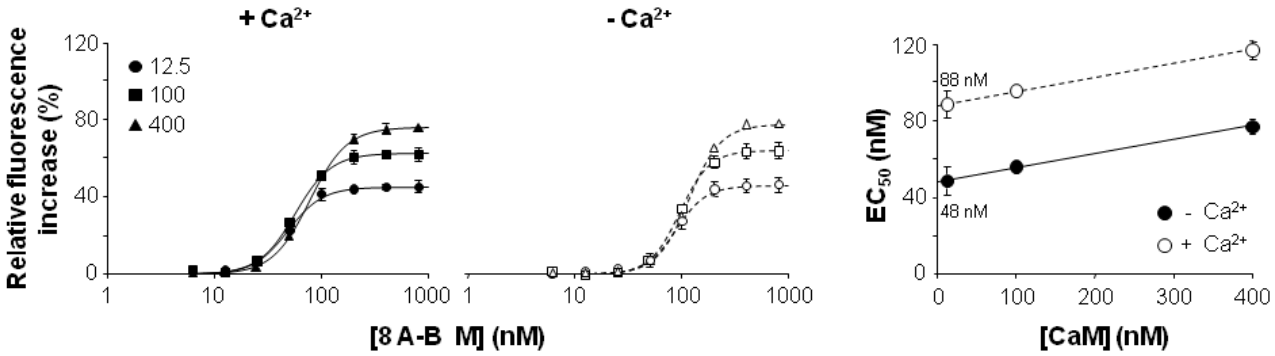



**Figure S4. Fluorescence analysis of peptide 8 binding to D-CaM.** Titration of D-CaM, at the indicated total concentration, in the absence (left, filled symbols) and presence (middle, open symbols) of 4 µM free $Ca^{2+}$. Right, extrapolation to obtain the Kd. The parameters used to fit a Hill equation to the data, using 12.5 nM fluorescent CaM, can be found in Table 1. The Kd values can be found in Table S1.

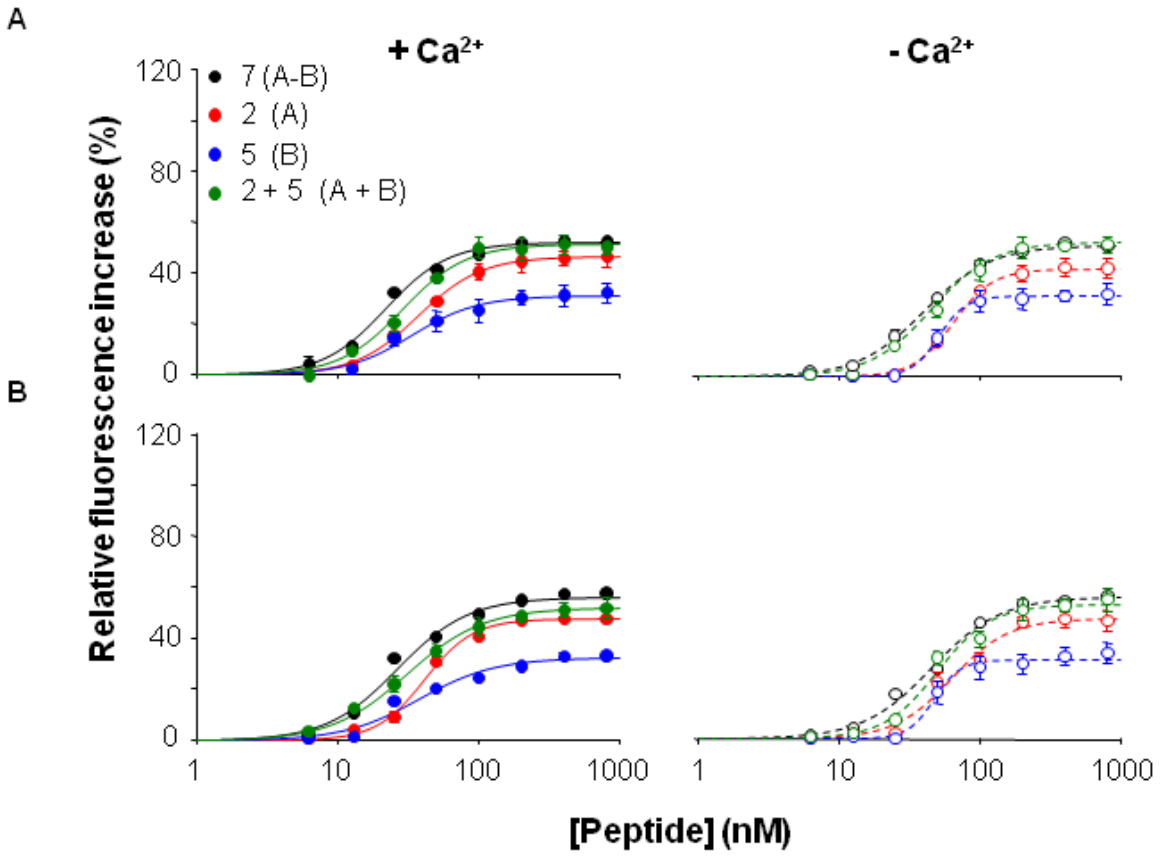



**Figure S5. Fluorescence titration of AEDANS-CaMs with peptide 2 and 5. A**) Relative increase in the emission fluorescence of 12.5 nM T34C AEDANS-CaM by peptide 2 (red), peptide 5 (blue) and an equimolar mixture of both peptides (green). The black lines are the best fits to the data obtained for the peptide 7. The experiments were performed in the absence (left, filled symbols) and presence (right, open symbols) of 4 µM frees $Ca^{2+}$. **B**) As in B, using T110C AEDANS-CaM. Data represent the means ± S.E. from three or more separate experiments. The parameters used to fit a Hill equation to the data, using 12.5 nM fluorescent CaM, can be found in Table 1.

**Table S1.** Summary of the binding parameters obtained using 12.5 AEDANS-CaMs.

| CaM | Peptide | - $Ca^{2+}$ (EGTA 10 mM) | | | + $Ca^{2+}$ (3.9 µM) | | |
|---|---|---|---|---|---|---|---|
| | | Maximal fluoresc. increase | $EC_{50}$ (nM) | h (n) | Maximal fluoresc. increase | $EC_{50}$ (nM) | h (n) |
| A34-CaM | **7** (A-B) | 51.7 ± 1.4 | 21.8 ± 1.7 | 1.9 ± 0.2 (3) | 50.7 ± 0.6 | 40.1 ± 1.3 | 1.8 ± 0.1 (3) |
| A110-CaM | **7** (A-B) | 56.0 ± 2.3 | 26.4 ± 3.3 | 1.7 ± 0.3 (3) | 55.6 ± 0.6 | 45.3 ± 3.2 | 1.7 ± 0.1 (3) |
| A34-CaM | **2** (A) | 46.1 ± 0.6 | 37.2 ± 1.2 | 1.9 ± 0.1 (3) | 41.8 ± 0.4 | 64.3 ± 1.4 | 3.1 ± 0.2 (3) |
| A110-CaM | **2** (A) | 47.2 ± 0.8 | 41.1 ± 1.8 | 2.5 ± 0.2 (3) | 47.3 ± 2.1 | 62.6 ± 6.5 | 2.1 ± 0.4 (3) |
| A34-CaM | **5** (B) | 30.9 ± 1.4 | 33.4 ± 4.1 | 1.8 ± 0.4 (3) | 30.9 ± 0.4 | 50.8 ± 1.1 | 4.5 ± 0.6 (3) |
| A110-CaM | **5** (B) | 32.4 ± 2.2 | 37.6 ± 7.3 | 1.6 ± 0.4 (3) | 31.1 ± 0.9 | 47.1 ± 2.4 | 4.6 ± 0.5 (3) |
| A34-CaM | **2** + **5** | 51.2 ± 1.5 | 28.6 ± 2.3 | 1.9 ± 0.2 (3) | 52.0 ± 1.6 | 45.1 ± 3.6 | 1.9 ± 0.3 (3) |
| A110-CaM | **2** + **5** | 51.9 ± 1.1 | 30.8 ± 1.8 | 1.6 ± 0.1 (3) | 52.8 ± 2.3 | 49.6 ± 5.2 | 2.3 ± 0.4 (3) |

**Table S2.** Kd values (nM) for PEG-conjugated peptides

| **CaM** | **Peptide** | **- $Ca^{2+}$ (EGTA 10 mM)** | **+ $Ca^{2+}$ (4 μM)** |
|---|---|---|---|
| D-CaM | **7** (A-B) | 20.9 ± 0.6 | 39.8 ± 0.9 |
| AEDANS-CaM T34C | **7** (A-B) | 22.9 ± 3.0 | 40.5 ± 2.1 |
| AEDANS-CaM T110C | **7** (A-B) | 24.6 ± 1.3 | 44.3 ± 0.2 |
| D-CaM | **2** (A) | 41.8 ± 0.5 | 69.1 ± 0.4 |
| D-CaM | **5** (B) | 28.5 ± 06 | 50.7 ± 0.3 |
| D-CaM | **2** + **5** | 26.6 ± 1.1 | 46.9 ± 0.5 |
| D-CaM | **8** (A-B M) | 48.4 ± 0.9 | 88.2 ± 0.7 |