**Supplemental materials**

**Supplement TABLE 1**
**Experimental restraints and structural statistics of the 30 NMR structures**

| | |
|---|---|
| Number of NOE cross-peaks found | 2249 |
| Number of NOE cross-peaks assigned | 2198 |
| Number of experimental restraints | |
| Upper distance restraints from NOE | |
| Total | 1022 |
| Short range | 474 |
| Medium range | 294 |
| Long range | 254 |
| Residual dipolar coupling restraints (H-N) | 89 |
| Dihedral angle restraints (TALOS) | 159 |
| Hydrogen bond distance restraints | 70 |
| $Ca^{2+}$-ligand distance restraints | 12 |
| Average r.m.s.d. values from experimental data | |
| Distance restraint violation (Å) | 0.052 ± 0.004 |
| Dihedral angle restraint violation | 0.323° ± 0.188° |
| RDC restraint violation (Hz) | 0.058 ± 0.016 |
| Average r.m.s.d. values from idealized covalent geometry | |
| Bonds (Å) | 0.0030 ± 0.0006 |
| Angles | 0.409° ± 0.021° |
| Impropers | 0.343° ± 0.020° |
| PROCHECK Ramachandran analysis for the folded region (residues 80-89) | |
| Residues in favored regions (%) | 94.1 |
| Residues in additional allowed regions (%) | 5.9 |
| Residues in generously allowed regions (%) | 0.0 |
| Residues in disallowed regions (%) | 0.0 |
| Coordinate precision of folded regions (residues 80-163) | |
| Backbone (Å) | 0.51 ± 0.08 |
| All heavy atoms (Å) | 1.06 ± 0.09 |

**Supplement TABLE 2**
**Rotational diffusion parameters for sCaM4-MKP1 and sCaM4ct-MKP1[a]**

| | | N-lobe (1-77)[b] | C-lobe (78-149) | C-lobe+MKP1 (78-166) |
|---|---|---|---|---|
| sCaM4-MKP1 | N | 43 | 49 | 56 |
| | $\tau_m$[c] | 8.73 ± 0.01 | 8.98 ± 0.01 | 9.04 ± 0.01 |
| | $D_{\|}/D_{\perp}$ | 1.39 ± 0.01 | 1.30 ± 0.01 | 1.30 ± 0.01 |
| | $\chi^2$ (red) | 13.80 | 24.40 | 31.15 |
| sCaM4ct-MKP1 | N | — | 55 | 66 |
| | $\tau_m$ | — | 6.03 ± 0.01 | 6.02 ± 0.01 |
| | $D_{\|}/D_{\perp}$ | — | 1.12 ± 0.01 | 1.13 ± 0.01 |
| | $\chi^2$ (red) | — | 18.85 | 18.00 |

[a] All values were obtained by fitting to the axially symmetric model.
[b] The solution structure of the N-terminal domain of $Ca^{2+}$-sCaM4 (PDB code: 2roa) was used for the fitting.
[c] The correlation time of molecular tumbling was calculated as $\tau_m = (6D_{iso})^{-1}$; $D_{iso}=1/3\ D_{\|}+2/3\ D_{\perp}$.

**Supplement TABLE 3**
**Summary of structural comparison to other CaM-target peptide complexes**

| Structure (PDB entry) | Backbone rmsd[a] | Difference in peptide angle[b] |
|---|---|---|
| RYR1 (2BCX) | 0.84 Å | 32.6º (3617-3638)[c] |
| smMLCK (1CDL) | 0.84 Å | 33.2º (798-813) |
| CaMKII (1CDM) | 0.80 Å | 32.2º (295-309) |
| CaMKI (1MXE) | 0.74 Å | 26.9º (298-317) |
| eNOS (1NIW) | 0.90 Å | 25.2º (496-506) |
| NMDAR (2HQW) | 0.91 Å | 34.2º (877-892) |

[a] Calculated for the well-folded region (residues 85-145).

[b] Calculated when the protein backbone atoms (residues 85-145) were superposed. The α-helical region of the MKP1 domain (residues 156-163) was used for the calculation.

[c] The α-helical region of the peptide used for the calculation.