Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Materials

Peptide synthesis

Peptides were synthesized using standard solid phase Fmoc chemistry protocol using a CS-Bio 136 automated peptide synthesizer (CS Bio Co., Menlo Park, CA) as described previously.[1] All peptides were amidated at their carboxy termini and acetylated at amino termini. The peptides were purified to homology (>95%) by high performance liquid chromatography (HPLC) and the molecular weights were identified by MALDI-TOF-MS (Autoflex III, Bruker Daltonics).

Circular Dichroism Spectra

Circular Dichroism Spectra (CD) were acquired at room temperature (RT) (Biologic MOS-450: 4.0 nm bandwidth, 0.1 nm resolution, 0.1 cm path length, 4.0 s response time and a 50 nm/min scanning speed). Peptide samples were incubated at 37°C for 30 min in phosphate buffered saline, pH 7.2 (PBS) and cooled to RT before measurement. The spectra were corrected by subtraction of the solvent blank. For thermal midpoint analysis, the temperature was controlled using a Bio-logic TCU250 system. The final concentration of N- and C-peptides was 1 μM in PBS. CD spectra were monitored at 222 nm between 20-90 °C.

Cell-cell fusion assays

Cell-cell fusion assays were performed as described previously.[2] HL2/3 cells which stably express HIV Gag, Env, Tat, Rev and Nef proteins (AIDS Reference and Reagent Program, NIH, form Dr. Barbara K. Felber and Dr. George N. Pavlakis)[3] and TZM-bl cells, which stably express

large amounts of CD4 and CCR5 (AIDS Reference and Reagent Program, NIH, from Dr. John C. Kappes, Dr. Xiaoyun Wu and Tranzyme Inc)[4], were used as the effector and target cells, respectively. TZM-bl cells ($2.5x10^4$ /well) and HL2/3 cells ($7.5x10^4$ /well) were co-incubated in 96-well plates (Corning Costar) at 37 °C in 5% CO_2 in the presence of different concentrations of inhibitors. After 6-8 h of incubation, the medium was aspirated and the cells were washed and lysed, followed by measurement of the luciferase activity using the Luciferase Assay System (Promega Corporation) on a plate reader (Molecular Devices SpectraMax M5). At least three repeated experiments were performed to derive the mean+/-SD of IC_{50}.

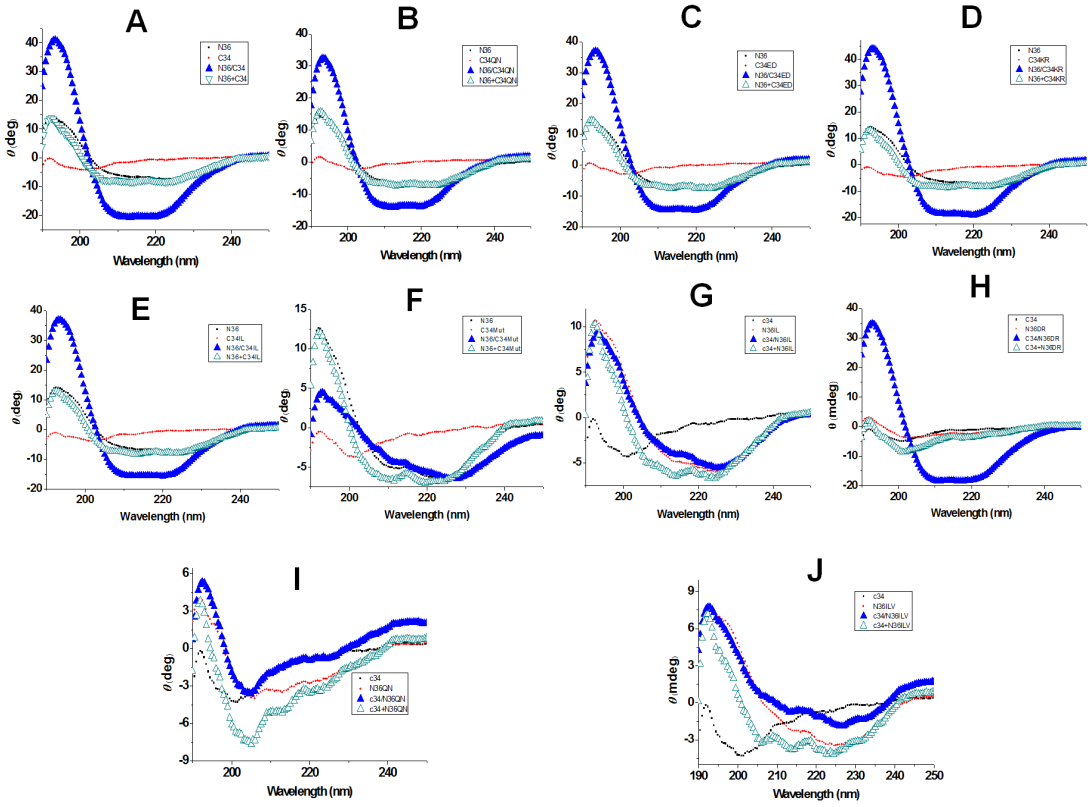

Figure S1 Interactions between mutated C34- or N36-peptides with N36 or C34 respectively. The circular dichroism (CD) spectra of isolated peptides were shown as small symbols, the mixture of the C34- and N36-peptides as large solid symbols, and the sum of the spectra of isolated peptides, indicating the non-interacting spectra of the two peptides, were shown as large open symbols. Differences between the spectra of the C34- and N36-peptides mixtures (large solid symbols) and the non-interacting spectra (large open symbols) indicate interactions between the two peptides.

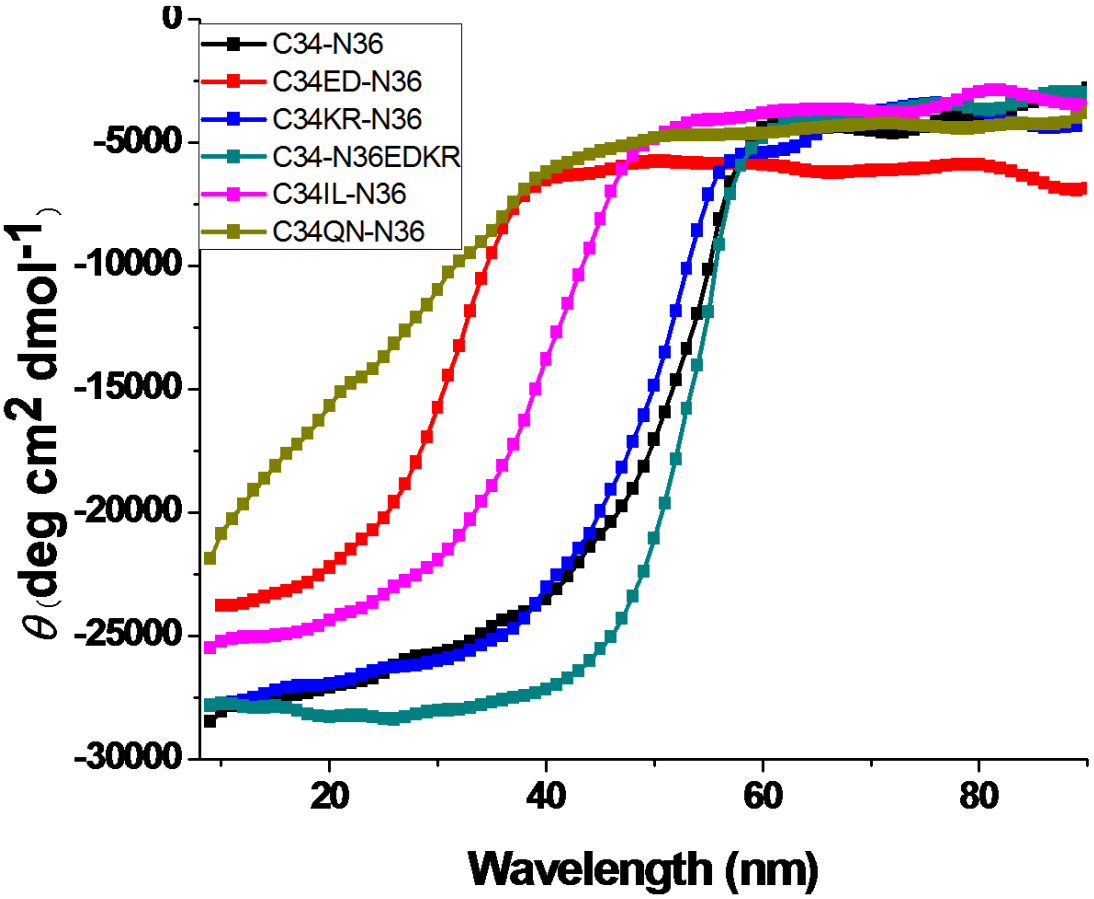

Figure S2 Thermal denaturation curve of six-helical bundle formed between C34- and N36-peptides. The CD thermal denaturations were monitored at 222 nm, and the melting temperatures (T_m) of six-helical bundles were shown in the inserted table. The C34QN-N36 didn't show a cooperative transition in the thermal denaturation curve for the *Tm* calculation, typical for a partly folded, molten globule state instead of a well folded protein.

References

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