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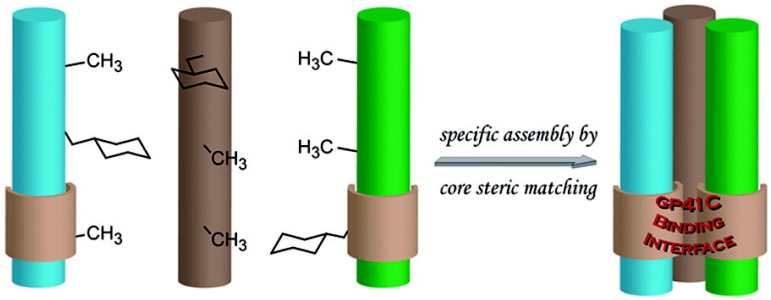

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Coiled-Coil Surface Presentation: An Efficient HIV gp41 Binding Interface Mimic

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The staggering destruction wrought by HIV infection has fueled a comparably vast quest for its containment and eradication. Recent work that illuminates its cellular entry mechanism has provided a new target in this struggle. Infectivity requires introduction of viral contents to the host cell, accomplished by fusion of cellular and viral membranes, mediated by the HIV envelope protein gp41. Central to this event is formation of a "trimer of hairpins" intermediate in which N-terminal sequences from three gp41 subunits form a coiled-coil whose exposed surface binds a C-terminal helix from each strand.[1] This interaction is critical for membrane fusion, and inhibitors derived from gp41 peptides or synthetic analogues can block infectivity.[2]

One barrier to facile development of molecules that bind the coiled-coil surface, and thus block the C-helix ligand, is the inconvenient behavior of the N-terminal coiled-coil when excised from its protein surroundings. Each face of the assembled trimer displays a deep hydrophobic pocket near one end, which is filled by a critical Trp, Trp, Ile trio of C-helix residues in the hairpin structure but which compromises proper folding and solubility in the stand-alone structure. Several approaches to well-defined gp41 mimics have been described, including attachment of additional solubilizing constructs and covalent tethering of multisubunit assemblies.[3] A potentially more straightforward approach involves populating two of the threefold symmetric trimer surfaces with charged, hydrophilic side chains, while retaining the native binding interface at the third. Such a design requires a 1:1:1 heterotrimer whose stoichiometry is controlled only by internal hydrophobic core residues. Previously, we have described steric matching of 2:1 alanine/cyclohexylalanine core layers for precisely this purpose.[4] Here we report application of our methods to construct an efficient model for the gp41 N-terminal trimer that requires no additional structural aids and effectively binds its C-terminal ligand.

The C-peptide binding interface of gp41 is primarily drawn from *b*, *c*, *e*, and *g* residues of the coiled-coil heptad repeat (*abcdefg*). Our designed complex grafts these side chains onto one of three surfaces of our previously reported T_9K:$T_{16}E$:$T_{23}E/K$ heterotrimer (Figure 1).[4b] One new peptide ($T_{16}HIV$) is obtained from $T_{16}E$ by replacing all *b* and *e* residues with those from gp41. Similarly, $T_{23}HIV$ is derived from $T_{23}E/K$ by installation of the *c* and *g* gp41 side chains, along with three key *f* residues. Together with T_9K, these two peptides can form a heterotrimer that contains favorable 2:1 alanine/cyclohexylalanine pairing at three consecutive *a* residues and matched Glu/Lys electrostatic contacts at two *e*/*g* interfaces. While these features ensure heterotrimer formation, the third interhelical surface (between $T_{16}HIV$ and $T_{23}HIV$) presents the C-peptide binding pocket.

Observed circular dichroism (CD) behavior supports the designed complex (Figure 2). The T_9K:$T_{16}HIV$:$T_{23}HIV$ trimer exhibits considerable helicity and characteristic minima at 208 and 222 nm. In contrast, gp41C, a 27 residue peptide encompassing the C-terminal helix sequence, is largely unstructured in isolation. When

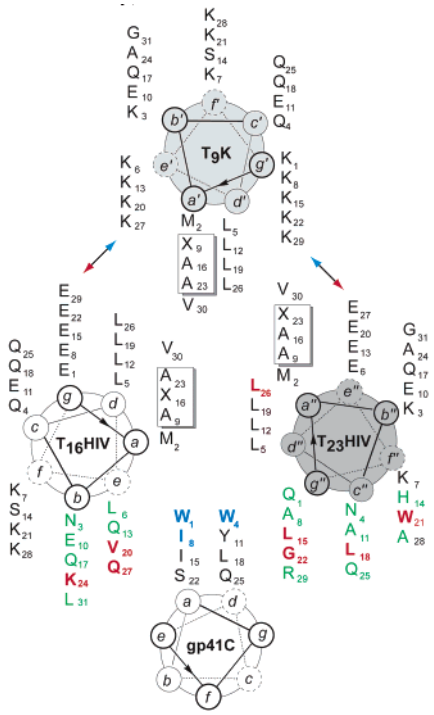

Figure 1. Heterotrimeric gp41 mimic. Complex of T_9K:$T_{16}HIV$:$T_{23}HIV$ forms specifically due to core matching of 2:1 alanine/cyclohexylalanine layers (boxes, X represents cyclohexylalanine). Electrostatic interfaces contain two Glu/Lys pairings (arrows) and one gp41C binding surface. Residues from gp41 are shown in green; those that form key binding contacts are in red. An abbreviated representation of the C-terminal ligand is also given, with critical Trp and Ile residues in blue.

mixed, the helicity of a 1:1:1:1 T_9K:$T_{16}HIV$:$T_{23}HIV$:gp41C solution is significantly in excess of the calculated weighted average component signals, indicating interaction of the peptides. Thermal unfolding experiments parallel these results, with both trimer and trimer−ligand complex displaying cooperative unfolding and high thermal stability (T_m = 85 and 83 °C, respectively).

To establish that HIV side chains were required for binding, a control complex was prepared by replacement of $T_{23}HIV$ with the parent sequence $T_{23}E/K$, eliminating half of the binding pocket and thus presumably abrogating its affinity for gp41C. Indeed, although a 1:1:1 T_9K:$T_{16}HIV$:$T_{23}E/K$ solution exhibits strong helicity by CD, addition of the ligand peptide diminishes the overall signal and affords a spectrum that precisely overlays the calculated weighted average for noninteracting species (Figure 3). Thermal

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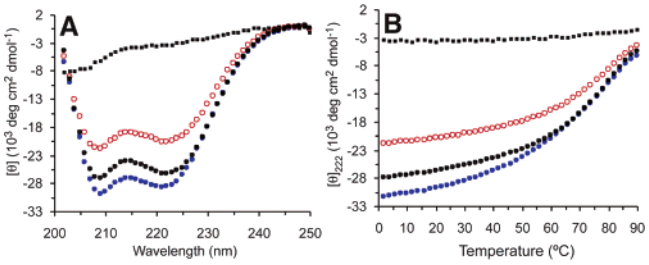

Figure 2. CD analysis of HIV mimic. Wavelength (A) and thermal unfolding (B) data for: gp41C (black squares), 1:1:1 T_9K:T_{16}HIV:T_{23}HIV (black circles), and an equimolar mixture of the T_9K:T_{16}HIV:T_{23}HIV trimer with gp41C (blue circles). Red open circles represent the calculated weighted average of pure trimer and ligand signals.

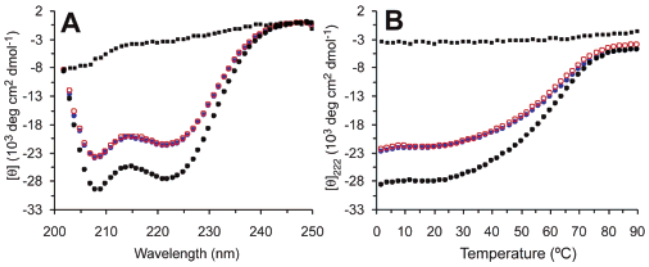

Figure 3. CD analysis of control complex. Wavelength (A) and thermal unfolding (B) data for: gp41C (black squares), 1:1:1 T_9K:T_{16}HIV:T_{23}E/K (black circles), and an equimolar mixture of the T_9K:T_{16}HIV:T_{23}E/K trimer with gp41C (blue circles). Red open circles represent the calculated weighted average of pure trimer and ligand signals.

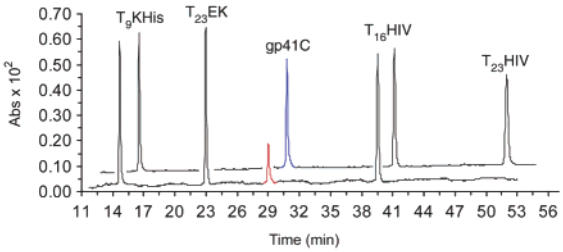

Figure 4. Ni−NTA analysis of HIV mimic. Control T_9K_{His}:T_{16}HIV:T_{23}E/K trimer (front trace) has dramatically lower affinity for gp41C (red) than the designed T_9K_{His}:T_{16}HIV:T_{23}HIV trimer (back trace, gp41C in blue).

unfolding spectra reveal similar results, and together these data support the unsuitability of the control trimer binding surface. Similar experiments on an equimolar T_{16}HIV:T_{23}HIV solution demonstrate that an intact trimer is required, as omission of T_9K prevents effective ligand binding.[5]

More direct evidence for assembly composition was obtained using previously described nickel-affinity methods and a Gly-Gly-$(His)_6$ tagged derivative of T_9K (T_9K_{His}). Elution fractions from parallel experiments suggest that the designed and control trimers have dramatically different affinities for gp41C, consistent with CD observations (Figure 4). While one equivalent of gp41C is retained from an initial 1:1:1:1 T_9K_{His}:T_{16}HIV:T_{23}HIV:gp41C mixture, the corresponding T_9K_{His}:T_{16}HIV:T_{23}E/K trimer binds only a fraction of the added ligand, providing strong evidence that the T_9K:T_{16}HIV:T_{23}HIV heterotrimer binds gp41C in the manner intended. Control experiments verified that each mixture forms 1:1:1 heterotrimers in the absence of ligand.[5]

Differential ligand affinities between designed and control heterotrimers are also reflected in analytical ultracentrifugation data (Table 1). Sedimentation equilibrium studies of a T_9K:T_{16}HIV:T_{23}HIV:gp41C mixture give an apparent relative molecular mass consistent with the ligand-bound trimer. The control complex, in contrast, affords a drastically lower value. Separate analysis of each individual trimer indicates that while the control species behaves as expected, the T_9K:T_{16}HIV:T_{23}HIV solution seems to contain some higher-order aggregates. Despite this result, the trimer CD signal is invariant over a broad concentration range, and performing the Ni−NTA analysis by first forming the trimer followed by subsequent ligand addition does not change the observed results.[5] Thus, these aggregation tendencies do not seem to interfere with ligand binding.

Table 1. Sedimentation Equilibrium Data[5]

sample	$M_{r,obsd}$	$M_{r,calcd}$[a]
T_9K:T_{16}HIV:T_{23}HIV:gp41C	14 208	15 159
T_9K:T_{16}HIV:T_{23}E/K:gp41C	9 345	15 310
T_9K:T_{16}HIV:T_{23}HIV	16 593	11 463
T_9K:T_{16}HIV:T_{23}E/K	11 560	11 614

[a] Calculated relative molecular mass for trimer or trimer plus ligand.

Together these data demonstrate that grafting of the HIV gp41 binding surface onto a well defined coiled-coil heterotrimer generates an efficient mimic capable of recognizing the native ligand. The model system is purely peptidic and requires no additional scaffolding to produce an effective mimic. The ability to control trimer stoichiometry by steric matching of core side chains should permit facile generalization of this strategy to encompass other viral systems with similar mechanisms.[2b]

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Supporting Information Available: Detailed experimental procedures, additional CD and Ni−NTA data, detailed analytical ultracentrifugation data, and helical wheel representations of control complex and designed complex with gp160 numbering. This material is available free of charge via the Internet at http://pubs.acs.org.

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(5) See Supporting Information for additional details.

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