JOURNAL OF CHEMICAL INFORMATION AND MODELING

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Peptide Inhibitors of Viral Assembly: A Novel Route to Broad-Spectrum Antivirals

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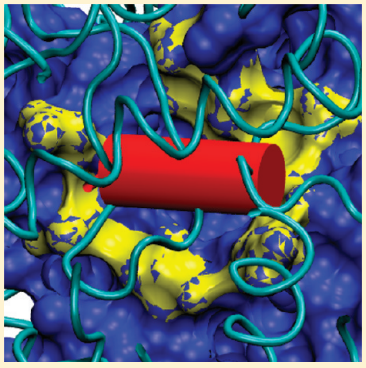
Supporting Information

ABSTRACT: We investigated the potential of small peptide segments to function as broad-spectrum antiviral drug leads. We extracted the α-helical peptide segments that share common secondary-structure environments in the capsid protein−protein interfaces of three unrelated virus classes (PRD1-like, HK97-like, and BTV-like) that encompass different levels of pathogenicity to humans, animals, and plants. The potential for the binding of these peptides to the individual capsid proteins was then investigated using blind docking simulations. Most of the extracted α-helical peptides were found to interact favorably with one or more of the protein−protein interfaces within the capsid in all three classes of virus. Moreover, binding of these peptides to the interface regions was found to block one or more of the putative "hot spot" regions on the protein interface, thereby providing the potential to disrupt virus capsid assembly via competitive interaction with other capsid proteins. In particular, binding of the GDFNALSN peptide was found to block interface "hot spot" regions in most of the viruses, providing a potential lead for broad-spectrum antiviral drug therapy.



INTRODUCTION

Viruses pose a threat to virtually every organism in every domain of life. With the urgent need for antiviral therapies for plant, animal and human hosts, research efforts have been focused on studying the structure−function relationships of viruses to support drug discovery and development. Recent advances in structure determination methodologies, for example, X-ray[1−3] and cryo-EM,[4−6] of viruses have led to large amounts of structural information at the atomic level. Structure-based phylogenetic analysis based on comparison of high-resolution data for the proteins forming the viral capsid shells has revealed unexpected similarities that are not visible in sequence comparisons of the capsid proteins or the viral genomes.[7] This has resulted in a regrouping of icosahedral viruses into a limited number of classes according to their capsid protein folds.[7] Each of these classes surprisingly covers several domains of life. For example; PRD1 and adenovirus belong to the same class despite infecting different hosts; eukarya and bacteria. This indicates that viruses affecting different domains of life can have capsid proteins that share the same fold even if the sequence similarity is very low. Clearly, such structural homologies among capsid proteins have ramifications for virology in general and for the development of antiviral drug inhibitors in particular.

The rapid increase in emerging virus pathogens has resulted in a pressing need for new antiviral therapeutics. For example, in the global human population, the emergence of 335 infectious diseases between 1940 and 2004 was reported, 25% of which are caused by viruses.[8] Many of those virus pathogens are the result of trans-species transmission,[9] for example, HIV-1[10] and SARS,[11] illustrating the need for broad spectrum antivirals that are effective across different species such as to intercept virus evolution. Moreover, due to the high mutation rates, especially in RNA viruses, the lifetime of specific antiviral therapeutics is often severely limited. Broad-spectrum antivirals would be a way of circumventing this problem. Recently, LI001 was described as an effective small molecule broad-spectrum inhibitor for numerous enveloped viruses including influenza and HIV-1.[12] LI001 disrupts cell entry via interaction with the viral lipid membrane.[12,13] However, it was found to have no effect on nonenveloped viruses where no lipid membrane exists, and the scope of this therapeutic is therefore severely limited. Selectively inducing apoptosis in cells containing viral RNA has provided another route to the development of broad spectrum antivirals[14] but is limited to dsRNA viruses. Modulation of the protein−protein interactions (PPI) within the virus capsids could be a more general route for the development of broad-spectrum antiviral drug candidates,[15] for both ssRNA and dsRNA enveloped and

Received: October 3, 2011
Published: March 6, 2012

nonenveloped viruses. Since distantly unrelated viruses share common capsid protein folds,[7] this suggests a novel route to a broad-spectrum antiviral: the possibility of disrupting virus assembly through inhibition of the (relatively preserved) capsid PPI by a molecular therapeutic. To our knowledge, this route has not yet been pursued and will be the focus of this work.

Development of protein−protein interaction inhibitors (PPII) is a challenge for conventional drug discovery largely due to the relatively flat topography of the protein−protein interfaces.[16] Classical drug discovery typically involves small molecules binding to deep pockets that are mostly hydrophobic in nature. However, scanning mutagenesis studies, in which amino acids at the protein surface are systematically mutated, have revealed that many protein−protein interfaces contain compact regions, termed "hot spots" (or anchor points) that are crucial for the affinity of the interaction.[16,17] Blocking these regions with small molecules, thereby, provides a potential target in the development of PPII lead structures.[18]

For the past two decades, use of small molecules has dominated drug development strategies[19] because of the technical difficulties in employing other biologically relevant molecular alternatives, such as short nucleotide segments[20] or small peptides.[21] Interest in the use of peptides as therapeutic agents was largely hindered because of the lack of progress in overcoming their susceptibility to in vivo degradation and elimination through the kidneys.[22,23] However, advances in peptide drug delivery protocols,[24−26] along with new techniques to enhance peptide structural stability,[27,28] have led to a growing interest in the use of peptides as drugs.[29,30] Peptides are interesting as therapeutic agents, as their properties span both small-molecule drugs and proteins and thereby lead to higher specificity and lower toxicity compared to the low molecular-weight compounds that are conventionally used in drug discovery. Peptides corresponding to helical segments are the most frequently considered in a therapeutic context, largely due to their relative ability to fold independently of other segments and thereby present a relatively well-preserved binding surface.[31] For example, it has been shown that peptides from one of the two extended helical domains of the HIV-1 transmembrane protein can block virion infectivity.[32] Other peptide mimics of the fusion proteins in retroviruses, filoviruses, coronaviruses, and herpesviruses have also been identified and shown to inhibit viral cell entry.[33−35] Moreover, peptides corresponding to helical segments of the gp41 protein have provided the basis for the development of an FDA-approved HIV-1 inhibitor, Fuzeon (Enfuvirtide), that prevents cell entry via disruption of virus assembly.[36]

In this work, we investigate the potential of small peptide segments as drug leads for the development of broad-spectrum antivirals. To achieve this, peptide segments were derived from the capsid protein structures that encompass three different virus classes: PRD1-like (PRD1, PBCV-1, and adenovirus), HK97-like (HK97 virus), and BTV-like (BTV, reovirus and RDV).[7] Although similar in terms of sharing the same capsid protein fold,[7] the pathogenicity of the different classes of virus spans a wide range of hosts from human (reovirus and adenovirus), animals (BTV virus), plants (RDF), and bacteria (PRD1 and HK97). The similarity of the structural features of the capsid proteins thereby opens up the possibility of finding a common mechanism for disrupting assembly (and thereby viability) for a broad spectrum of viral pathogens. The approach we adopt is to identify short peptide segments extracted from analogous capsid protein structural features that could act to disrupt capsid protein interfaces via competitive binding. To assess this, we carried out blind docking[37] of all α-helical segments that share a common secondary-structure environment in the capsid protein−protein interfaces for viruses within the three classes. The binding affinity of the peptides at the interface regions of the different capsid proteins was then assessed, primarily in terms of the capacity to block putative PPI hot spots. The identification of peptides whose binding to capsid proteins disrupts assembly in all of the viruses considered thereby provide a route for the discovery of potential novel broad-spectrum peptide-based antiviral drug leads.

MATERIALS AND METHODS

The X-ray crystal structures of the full virus capsids of viruses belonging to the PRD1-like, HK97-like, and BTV-like classes, as identified by Bamford et al.,[7] were retrieved from the VIPER database.[38] These comprise, for the PRD1-like class PRD1, PBCV-1, and adenovirus (PDB ids 1w8x, 1m4x, and 3kic); for the HK97-like class HK97 (PDB id 1ohg); and for the BTV-like class BTV, reovirus and RDV (PDB ids 2btv, 1ej6, and 1uf2).

Identification and Extraction of Capsid Interface Regions. For each capsid protein, interfaces to neighboring proteins within the virus capsid were extracted on the basis of proximity within a distance threshold of 9 Å using CA atoms. The use of a 9 Å threshold allowed for some preservation of the integrity of the secondary structure elements of the complementary interfaces. The secondary structure of both sides of the interface was then determined using the DSSP program.[39] With our focus on α-helical secondary structure elements, any interfaces containing turns, coils, or both were further split into multiple interface segments by removal of these regions.

Coding Capsid Interfaces via Complementary Secondary Structure Elements. Each interface was then labeled by mapping the secondary structure of each residue on one side of the interface to its nearest neighbor on the complementary interface side. In this process, starting at one side of the interface, each residue was labeled by upper case letters indicating its secondary structure, for example, H for a helix and E for an extended sheet. Each of these labels was then affixed by the secondary structure label of its nearest neighbor residue on the complementary interface in lower case letters, for example, h for a helix and e for an extended sheet. This resulted in a compact coding of the interface that collectively represents the secondary structure elements on both sides. For example, an HhHhHhTt complementary secondary structure coding of an interface indicates that the first three residues that are part of an α-helix on one side (HHH) are nearest neighbors to three residues that are part of an α-helix on the other side (hhh) while the fourth residue is part of a turn on both sides (T and t). It is noted that coding the interfaces in this way transforms the problem of finding common interfaces from a computationally intensive three-dimensional geometrical search to a one-dimensional string matching problem.

Identification of Peptide Segments from Analogous Local Secondary Structure Environments at Capsid Interfaces. The longest helical peptides that share common complementary secondary structure interfaces across the seven viruses considered were then selected. The common complementary secondary structure interfaces of the selected peptides were found to be helical in most cases. The selected peptides

correspond to 12 ocatapeptide segments; WHLHFVNT, GDFNALSN TAAIARAA, LLLSTLAD, AEHRAMIQ, VIYSMLND, GLRTAIRN, RDMMRWVL, DHVYNYMT, QNMARQLD, NDVRKIYL, and HLSLMQRR, which were then retained for further analysis.

Blind Docking of Peptide Segments to Virus Capsid Proteins. Blind docking[37,40] of the selected peptide segments was then carried out against all of the proteins of the asymmetric units of the seven virus capsids using the AutoDock 4.1 suite of programs[41] (a total of 1131 blind docking simulations for each peptide segment; see Table 1 for details).

Table 1. Details of the Docking Simulations Conducted for the PRD1-like, HK97-like, and BTV-like Virus Classes

virus class	virus	PDB id	number of proteins/ capsid asymmetric unit	number of protein−protein interfaces	total number of docking simulations[a]/ single peptide ligand
PRD1-like	PRD1	1w8x	15	124	216
	PBCV-1	1m4x	3	19	48
	adenovirus	3kic	1	9	24
HK97-like	HK97	1ohg	7	90	144
BTV-like	BTV	2btv	15	131	252
	reovirus	1ej6	5	33	150
	RDV	1uf2	16	139	297

[a]Total number of docking simulations for a single peptide = the sum of the number of the grid cells around each of the proteins of the capsid asymmetric unit.

Computation of the potential energy grids required by AutoDock at appropriate resolution is quite demanding for large proteins in terms of memory storage and CPU time; therefore, the space around each protein was partitioned using a grid that encompasses the whole protein and extends outward by 40% of its extent in each direction. A grid cell extent of 64 Å was used throughout. For each grid cell, the grid potentials were computed at 0.5 Å resolution for the docking calculations. To alleviate the possibility of missing binding sites that lie across neighboring grid cells, two overlapping grids were used that are shifted by 20 Å in each direction. We note that partitioning the space around the protein into grid cells that are treated independently by AutoDock serves two purposes: first, it circumvents the prohibitive computational cost of carrying out blind docking to the whole of each protein in a single run; second, since the same grid cell extent is used throughout, this setup maintains a consistent ratio of the number of docking simulations to protein size, which varies across the seven viruses. In all of the docking simulations the protein was kept rigid and fixed in space while the peptide was placed at a random initial position and orientation. In the peptide docking, a search in the space surrounding the protein was performed using the Lamarckian Genetic Algorithm (GA-LS)[42] with the backbone torsion angles of the peptide segments kept fixed, while the side chain torsion angles were assigned initial random values and allowed to change conformation. For every arbitrary starting position of the peptide, 20 hybrid GA-LS docking runs were carried out using a population size of 200, a maximum number of energy evaluations of 3 000 000, a maximum number of generations of 27 000 and 300 iterations of local search. The structures of the docked peptide segments were stored and subsequently checked for overlap with the interface regions of each capsid protein. Peptides that overlap with at least three residues of the interface of neighboring proteins, using a 3.0 Å distance threshold between the CA atoms, were selected and the peptide−protein complex with the lowest binding free energy was retained in each case.

To explore the utility of our peptide selection methodology, blind docking simulations were repeated using three randomly selected helical peptides extracted from unrelated proteins as negative controls. The control peptides were extracted from the epidermal growth factor receptor (PDB id 2j6m; RES id 459:766), Hemoglobin alpha chain (PDB id 1b86; RES id 96:103), and MCP-1 protein (PDB id 3ifd RES id 63:70).

RESULTS AND DISCUSSION

Competitive binding of small molecules with individual proteins at the interface of a protein−protein complex underpins the design and development of PPI inhibitors.[16−18] The difficulties encountered in disrupting a single protein−protein interface are exacerbated when considering binding at *multiple* protein−protein interfaces, for example, capsid protein interfaces across a range of viruses. To tackle this challenge, we adopt a three stage approach: (1) identification of candidate ligands that could interact with the capsid proteins of different viruses, (2) identification of possible binding modes of these ligands with all capsid protein targets, and (3) assessment of the potential of a *single* ligand to disrupt capsid assembly of *multiple* viruses, that is, identification of a potential broad-spectrum antiviral drug lead.

Stage 1: Capsid Protein−Protein Interface As a Source of Candidate Peptides. Because of the large number of protein−protein interfaces in the different virus capsids (a total of 545 interfaces; see Table 1 for details), conventional screening of large small molecule ligand libraries against capsid protein targets could easily become computationally intractable. We adopted a different approach, focusing on peptide ligands. Peptides are structurally native to the protein−protein interfaces and possess all short-range interactions that are essential for the stabilization of protein−protein interactions. This endows them with specificities and hence makes them strong candidates for competitive binding with endogenous protein partners. Using peptides thereby restricts the subset of the myriad chemical structures that could be considered as candidate ligands. Further, we restrict our search to α-helical peptide segments that are known to fold independently to form stable structures. These can be further stabilized by chemical modification, for example, stapled peptides.[43,44] In this framework, the initial screening problem becomes one of identifying the appropriate α-helical peptide sequence.

Our approach was to co-opt the structural elements observed at the protein−protein interfaces within the seven virus capsids. We extracted the protein−protein interfaces from the structures of all seven virus considered and retained only segments that correspond to α-helical peptides. As a heuristic selection criterion, we retained the longest α-helical peptides that share the same secondary structure environment in all of the seven viruses, reasoning that this would enhance specificity. This approach led to the identification of a small library of twelve octapeptides: WHLHFVNT, GDFNALSN TAAIARAA, LLLSTLAD, AEHRAMIQ, VIYSMLND, GLRTAIRN, RDMMRWVL, DHVYNYMT, QNMARQLD, NDVRKIYL, and HLSLMQRR. All of these α-helical peptide segments were found to interact with α-helical aggregates at the capsid

dx.doi.org/10.1021/ci200467s | J. Chem. Inf. Model. 2012, 52, 770−776

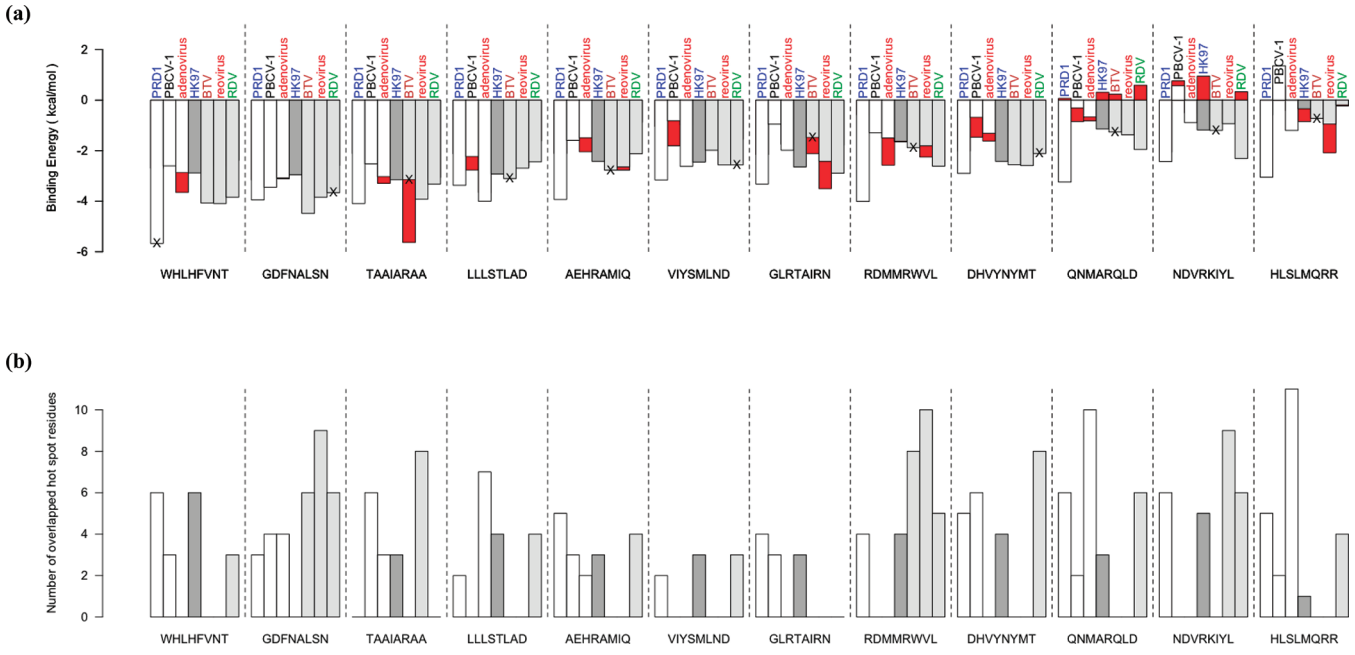


Figure 1. (a) Lowest binding energy of the peptide−protein complexes that overlap with the protein−protein capsid interfaces in the three virus classes PRD1-like (white), HK97-like (dark gray) and BTV-like (gray) for twelve α-helical peptide segments (bottom labels). The lowest binding energy of peptide−protein complexes away from the interface regions are shown in red (background barplot) for each virus. Viruses within each class are labeled at the top of each bar and colored according to their domain of pathogenicity; human (red), bacteria (blue), plant (green), unicellular organisms (black). The virus from which each peptide is extracted is labeled by an "X". The corresponding number of interface "hot spot" residues that overlap with the peptide in each case is shown in (b).

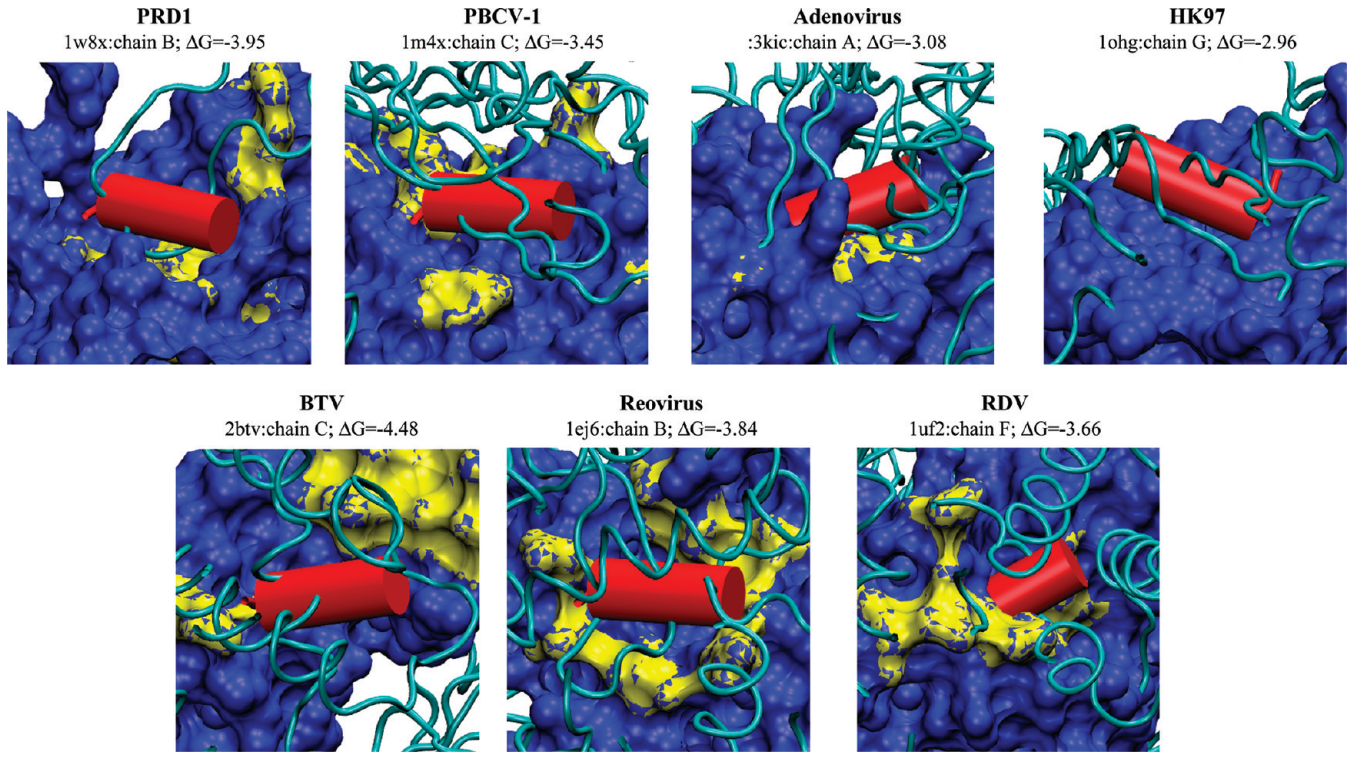


Figure 2. Lowest energy α-helical GDFNALSN peptide (cartoon representation; red) docked to capsid proteins (surface representation; blue) of the PRD1, PBBCV-1, adenovirus, HK97, BTV, reovirus, and RDV. Hot spot regions within each capsid protein surface are shown in yellow while neighboring chains in the corresponding virus capsid are shown in tube representation in each case. Capsid protein PDBID, its chain identifier and the corresponding binding energy (ΔG in kcal/mol) are indicated on top of each panel.

protein−protein interfaces. We note that these peptides are not related in terms of their location within the individual capsid sequences.

Stage 2: Identifying Possible Binding Modes and Interactions to All Capsid Protein Targets. Since the peptide segments were extracted from different viruses, their

dx.doi.org/10.1021/ci200467s | J. Chem. Inf. Model. 2012, 52, 770−776

binding sites across the full range of capsid protein surfaces considered were not known a priori. We therefore carried out blind docking for each peptide against all of the capsid proteins in order to assess their binding mode and affinity. This revealed that potential binding sites are widely distributed over the surface of the capsid proteins. However, our focus was on preferential binding at the capsid protein interface regions as these have the potential to disrupt capsid assembly. Interestingly, many of the peptide segments exhibit binding energies that are more favorable at the interface regions compared to the rest of the protein surface (Figure 1 a). In particular, the GDFNALSN peptide exhibits preferential binding at the interface regions in all seven virus capsids under investigation. Other peptides, for example, WHLHFVNT, also show similar preferential binding to the interface regions, albeit somewhat less prominent for the adenovirus. Ten out of the twelve peptides show favorable binding energy across the seven viruses (Figure 1a).

Stage 3: Assessment of Potential Disruption of Capsid Protein Assembly Across *Multiple* Viruses by a Single Peptide. Our strategy requires not only that peptides bind favorably to interface regions but also that they offer competitive binding to other capsid proteins across the three classes, thereby disrupting virus assembly. To assess this, we need to compare the relative binding affinities of the peptides to that of the corresponding capsid protein pairs in each virus. Unfortunately, experimental association energy data is not available for every interacting protein pair within each virus. Moreover, computational estimation of the association energy of such a large number of protein pairs (Table 1) is not straightforward in terms of the accurate estimation of the various energetic and entropic components[45−47] and can be computationally demanding, depending on the approach taken. For example, the VIPER database[38] reports the capsid protein−protein association energies for most viruses based on the buried surface areas and atomic solvation parameters. This energetic approach (and associated parameters) has a difference of focus and scale compared to that used for estimating ligand binding affinities by AutoDock, which significantly complicates any attempt at comparing relative binding energies. Considering both classes of binding partners in the same energetic framework would add considerably to the complexity and effort of our approach.

To circumvent these problems, we adopted a heuristic that relies on peptide being a competitive binder if its binding mode coincides a protein−protein association hot spot, a compact region of the interface that significantly contributes to its binding affinity with other protein partners.[48] We therefore computed the location of these hot spots using the HotPoint webserver[49,50] and examined their overlap with the binding mode of the docked peptides (Figure 1b). The GDFNALSN peptide overlaps with the hot spot regions in six out of the seven viruses investigated (Figure 2) and therefore its competitive binding with the proteins forming the capsid of these viruses is likely to disrupt capsid assembly in all of them. Similar behavior can also be observed for other peptides but in these cases the viral coverage is lower. Interestingly, none of the three random peptides used as negative controls (see Material and Methods), showed overlap with the hot spot regions across the seven viruses considered (Supporting Information Figure 1).

SUMMARY AND CONCLUSIONS

We exploited the structural similarity of capsid proteins across three different virus classes[7] to investigate the potential for broad-spectrum antiviral peptide drugs that act by disrupting virus assembly via competitive binding at capsid protein−protein interfaces. Candidate α-helical peptide segments were extracted from analogous structural features of capsid protein−protein interfaces. Blind docking of each such peptide to the virus capsid proteins covering the different virus classes revealed favorable binding affinity to at least one of the protein−protein interface regions in all of these viruses. Moreover, certain candidate peptides, e.g. the GDFNALSN and WHLHFVNT octapeptides, were shown to have favorable binding modes that block hot spot regions at the protein−protein interfaces in most of these virus capsids. GDFNALSN in particular is characterized by the absence of significant binding affinity to any area on the capsid proteins other than the protein−protein interfaces, making it especially suitable to target those areas. The binding affinities of GDFNALSN to capsid proteins in all virus classes considered here are comparable to the affinity to RDV from which it has been isolated. This suggests that it has the potential to outcompete the binding of native partner capsid proteins of these viruses during assembly. Given that only a limited number of the protein−protein interfaces within the capsid need to be disturbed in order to inhibit the assembly of a functional viral particle, competitive binding of the α-helical peptide segments with individual capsid proteins should be sufficient to disrupt virus capsid assembly in all of the virus classes considered here. This effect could potentially be enhanced further, for example, using stabilization of the peptides by chemical modification, e.g. through stapling,[43,44] to optimize the binding properties of the peptides. As helical interactions are common and widespread functional motifs[51,52] in protein−protein interaction this might result in a lack of specificity of the peptides for their capsid protein interface targets and could also lead to toxicity issues; selective chemical modification could be away to alleviate such problems. We acknowledge that we use a number of heuristics that have been carefully considered to keep the approach physically realistic, while computationally tractable, and that alternative approaches e.g. in terms of peptide sequence selection, or using more sophisticated and costly energetic considerations are possible and could also be considered in further work. In conclusion, we introduce an approach to identifying peptides that have the characteristics to inhibit capsid protein−protein interactions across human, animal and plant viruses and bacteriophages. The motivation for this approach is the disruption of the viral assembly process, whose thermodynamic, kinetic and functional requirements may give rise to structural constraints that constitute an alternative angle of attack (or Achilles' heel) for antiviral therapy. The peptide segments discovered in this work are candidate capsid protein−protein interaction inhibitors that we suggest might serve as leads for the development of broad-spectrum antiviral drugs.

ASSOCIATED CONTENT

Supporting Information

Figure 1 (a) The lowest binding energy of the peptide−protein complexes that overlap with the protein−protein capsid interfaces in the three virus classes PRD1-like (white), HK97-like (dark gray) and BTV-like (gray) for three unrelated randomly selected peptides (bottom labels pdbid_1st_resi-

dx.doi.org/10.1021/ci200467s | J. Chem. Inf. Model. 2012, 52, 770−776

d_last_resid). The lowest binding energy of peptide−protein complexes away from the interface regions are shown in red (background barplot) for each virus. Viruses within each class are labeled at the top of each bar and colored according to their domain of pathogenicity; human (red), bacteria (blue), plant (green), unicellular organisms (black). The corresponding number of interface "hot spot" residues that overlap with the peptide in each case is shown in (b). This information is available free of charge via the Internet at http://pubs.acs.org

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work has been supported by a Research Leadership Award from the Leverhulme Trust to RT. We are very grateful to Drs. Garib Murshudov and Seishi Shimizu for provision of computing resources.

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