

Using Modeling and *de novo* Peptide Design to Identify Novel, Optimizable, Broad-Spectrum and Specific Flavivirus Anti-Infectives: From Concept to Success in Eight Weeks

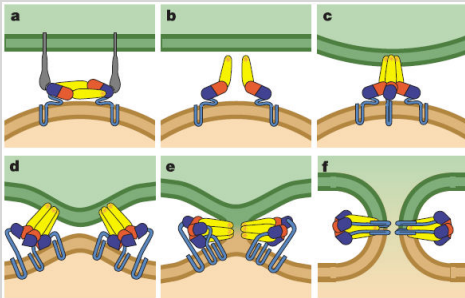
Joseph Audie[‡], Michel Ledizet[§], Ray A. Koski[§] and Nathalie Bonafé[§]

[‡] CMBioscience, Science Park, New Haven Connecticut, [§] L2 Diagnostics, LLC New Haven CT

Introduction

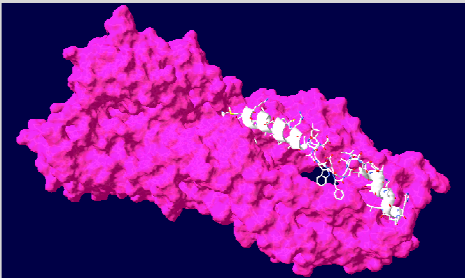
The Flavivirus genus includes the West Nile virus (WNV), dengue virus (DV), Tick-borne Encephalitis Virus, Yellow Fever Virus, and several other viruses. WNV and the four serotypes of DV are especially significant in terms of human pathogenicity and are classified as priority pathogens by the National Institute of Allergy and Infectious Disease (NIAID). There is a pressing need for the development of molecular therapies to treat WNV and DV infection. Ideally, a single broad spectrum compound, effective against WNV and all four DV serotypes, will be identified. We used a recently solved crystal structures of the DV1 envelope protein, protein modeling, and *de novo* structure-based peptide design to identify 27 tetra-peptides that were predicted to block a key intra-protein interaction between the envelope protein stem peptide and the larger envelope protein. Importantly, the stem region we targeted is highly conserved across all DV serotypes, WNV and other flaviviruses. The entire computational phase of the project took 8 weeks. All 27 tetra-peptides were synthesized and tested for their ability to neutralize WNV, DV 1 and DV2 in cell-based assays. Five of the tetra-peptides inhibited WNV, DV 1 and DV2 infectivity in Vero cells. Testing of the five tetra-peptides for inhibition of viral entry of unrelated viruses produced negative results, suggesting they are likely to inhibit entry of flavivirus by a specific mechanism. Thus, the data suggests that within 8 weeks we used protein modeling and structure-based *de novo* peptide design to identify five novel and optimizable tetra-peptides that are specific inhibitors of flavivirus infectivity worthy of continued development. Our results are all the more impressive given that past research efforts that employed more conventional biological and chemical approaches failed to yield any promising peptides. Future work will focus on optimizing the tetra-peptides into more drug-like lead compounds.

Flavivirus Viral Entry Model



Viral entry into host cells depends on specific intramolecular rearrangements in the DV envelope protein. Specifically, in panel e the transmembrane domain is shown making contact with the stem domain, leading to a rearrangement that brings about membrane hemifusion.

Dengue 1 Stem Peptide Model



We used structure of the Dengue virus type 1 envelope protein in the post-fusion configuration (PDB 3G7T) to model the structure of generalized flavivirus envelope proteins.

Key experimental data

We have modeled interactions between a conserved region of the Dengue Envelope protein and the designed peptides to identify novel compounds that block viral entry into mammalian epithelial cells. Key data includes:

- Crystal structure of the Dengue Virus envelope protein
- Predicted binding energies of three rounds of designed tetrapeptides
- Specific predictions of the protein-peptide interaction surfaces
- Cell-based assay results: 5 peptides that significantly reduce infectivity, measured for both Dengue and West Nile Virus
- The technology is substantially more efficient than phage display technologies for identifying peptide sequences that bind to specific targets.

CMBioscience Proprietary Structure-Based Peptide Discovery Platform

CMDdock: our proprietary protein-peptide docking work-flow for accurately predicting and modeling protein-peptide interactions

CMDScreen: our proprietary protein-peptide virtual screening work-flow for quickly and efficiently identifying promising peptide hits

CMDDesign: our proprietary *de novo* peptide ligand design work-flow for engineering truly novel peptide hits

CMDScore: our patented empirical scoring function for quickly and accurately estimating protein-protein and protein-peptide binding affinities

CMDscore: our proprietary force-field based methodology for quickly and accurately estimating protein-protein and protein-peptide binding affinities

CMDsolvation: our proprietary empirical method for quickly and accurately estimating peptide water-octanol transfer free energies

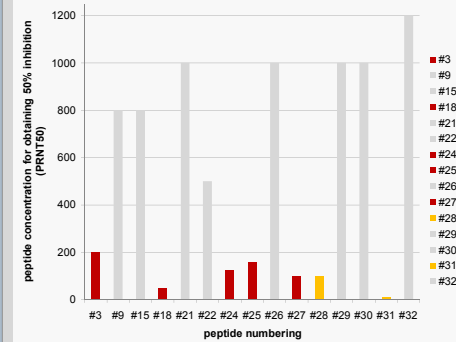
CMDbuild: our proprietary computational work-flow for analyzing, building, modeling and validating protein and peptide structures

Our empirical formula for determining free energy of binding:

$$dG_{bind, empirical} = -0.79 \Delta X_{+/-} + 0.075 \Delta X_{c/l/s} - 0.65 X_{ab} - 0.86 X_{ab} - 0.00089 X_{gap} - 0.089 \Delta X_{sur} - 0.33$$

Cell-based Assay Testing Results for West Nile Virus

We synthesized all 27 design predictions and tested them in a cell-based assay that indicates that 5/27 designed peptides are \approx μ M inhibitors of Dengue infectivity. These small, effective and optimizable compounds can serve as the starting points for small molecule peptide mimetics or can serve as the basis for subsequent rounds of modeling.



Positive controls (30-residue peptide known to inhibit infectivity) is in yellow. Successful designed peptides are in red. Unsuccessful peptides are in grey.

Results: Peptide Predicted Binding Energies

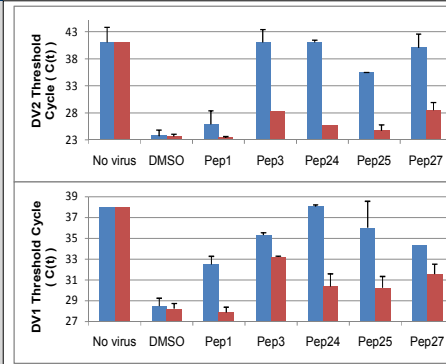
Solution	CMDscore dG (kcal)	CMDScore dG (kcal)	CMDdock dG (kcal)
1.1	-15.0	-9.7	-7.9
1.2	-15.6	-9.7	-6.6
1.3	-15.2	-9.6	-8.2
1.4	-15.7	-9.8	-8.0
1.5	-20.5	-5.1	-5.9
1.6	-15.6	-9.5	-8.8
1.7	-15.3	-9.5	-6.8
1.8	-15.2	-9.5	-7.0
1.9	-15.6	-9.8	-7.9

The top 27 high-scoring peptides from the first three rounds of peptide design. These peptides were selected from the 20⁶ (160,000) possible tetrapeptides based on their predicted binding energies as calculated by CMDscore, CMDScore, and CMDdock. In addition, each peptide was considered in 3 poses, for a total of 480,000 binding-energy predictions.

Solution	CMDscore dG (kcal)	CMDScore dG (kcal)	CMDdock dG (kcal)
2.1	-19.6	-4.9	-7.4
2.2	-17.5	-5.6	-5.5
2.3	-19.9	-4.8	-6.5
2.4	-20.5	-5.1	-6.2
2.5	-20.3	-4.9	-4.3

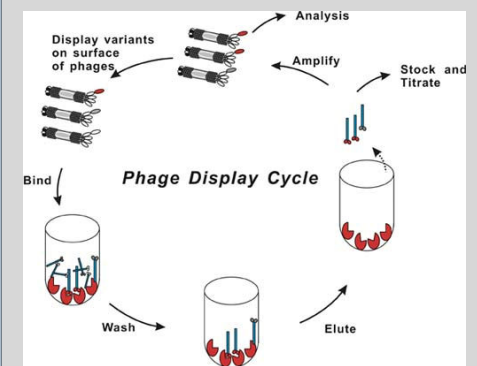
Solution	CMDscore dG (kcal)	CMDScore dG (kcal)	CMDdock dG (kcal)
3.1	-18.7	-4.2	-5.8
3.2	-17.9	-4.0	-6.2
3.3	-17.8	-4.3	-7.5
3.4	-17.8	-4.3	-8.0
3.5	-17.8	-4.0	-5.8
3.6	-17.7	-4.1	-6.9
3.7	-17.6	-4.3	-7.7
3.8	-17.4	-4.0	-7.8
3.9	-17.4	-4.3	-7.7
3.10	-17.4	-4.2	-7.6
3.11	-17.2	-4.1	-7.7
3.12	-17.1	-4.3	-6.3
3.13	-16.2	-4.0	-7.3

Cell-based Assay Results for Dengue 1 and 2



Quantitative PCR assays show that peptides are effective against both West Nile Virus and two isotopes of Dengue virus. These results are a strong indication that the region of flaviviruses targeted by the peptides is important for all flaviviruses, and that the peptides are capable of inhibiting a broad spectrum of flaviviruses.

Advantages over Phage Display technologies



CMBiosciences uses technology that is substantially more efficient than phage display technology, our closest competitor. The most important is that we do most of our iterative calculations in silico at very fast rates and very low cost, allowing you to move to the analysis step directly. Second, phage display is limited to those sequences that the host bacteria can fold and export, which means that the full peptide design space is not exploited. Our technology is therefore much faster as well as a more complete search of the available peptides. Third, we can use non-standard amino acids and even fluorescent tags as part of our in silico searches, an option not offered by phage display at all.

Conclusion

CMBioscience peptide design, docking, and scoring algorithms were successfully used to design a series of peptides that bind to the flavivirus stem regions and inhibit infectivity of multiple flaviviruses as measured in a cell-based assay using Vero cells. The computational portion of this challenging design problem was accomplished in only eight weeks from start of project to delivery of peptides. We believe that this methodology can be used to design peptide inhibitors of a large number of viruses in the flavivirus family. The implications for wider use are that a great many intra- and inter-protein interactions important for pathogenicity can be targeted in an efficient manner using CMBioscience tools.

References

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- 2: Audie J, Scarlata S. A novel empirical free energy function that explains and predicts protein-protein binding affinities. *Biophys Chem* 2007;129:198.
3. Modis et al. 2004. Structure of the Dengue Virus Envelope Protein After Membrane Fusion. *Nature* 427:313-319.

Contact information

Joseph Audie

Sacred Heart University
5151 Park Ave.
Fairfield, CT 06825

E: audiej@sacredheart.edu
audiej@sacredheart.edu