

Design of small-molecule inhibitors for the Bug to Drug™ Program*

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Abstract

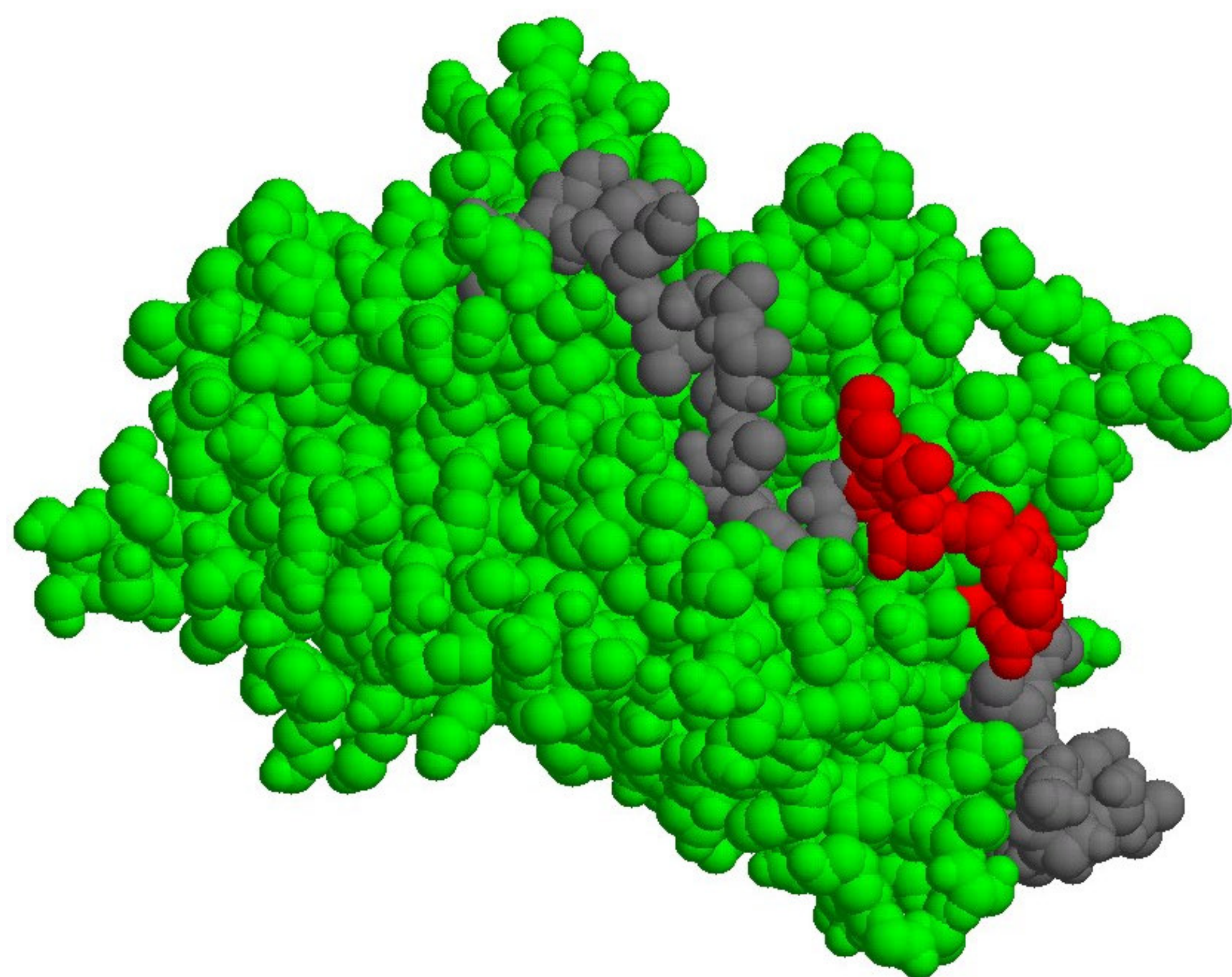
Sarnoff Corporation is conducting a “Bug to Drug™ Identification and Countermeasures Program” for the U.S. Government. The goal of this program is to develop the enabling technology to allow for an extremely rapid response to a bio-warfare threat. An important element of this program is the design of small-molecule inhibitors to toxins which might be used in bio-warfare. These inhibitors are designed computationally using proprietary algorithms. The goal of this program is twofold: to demonstrate the technology today and to improve it (in both speed and efficacy) for the future.

Background

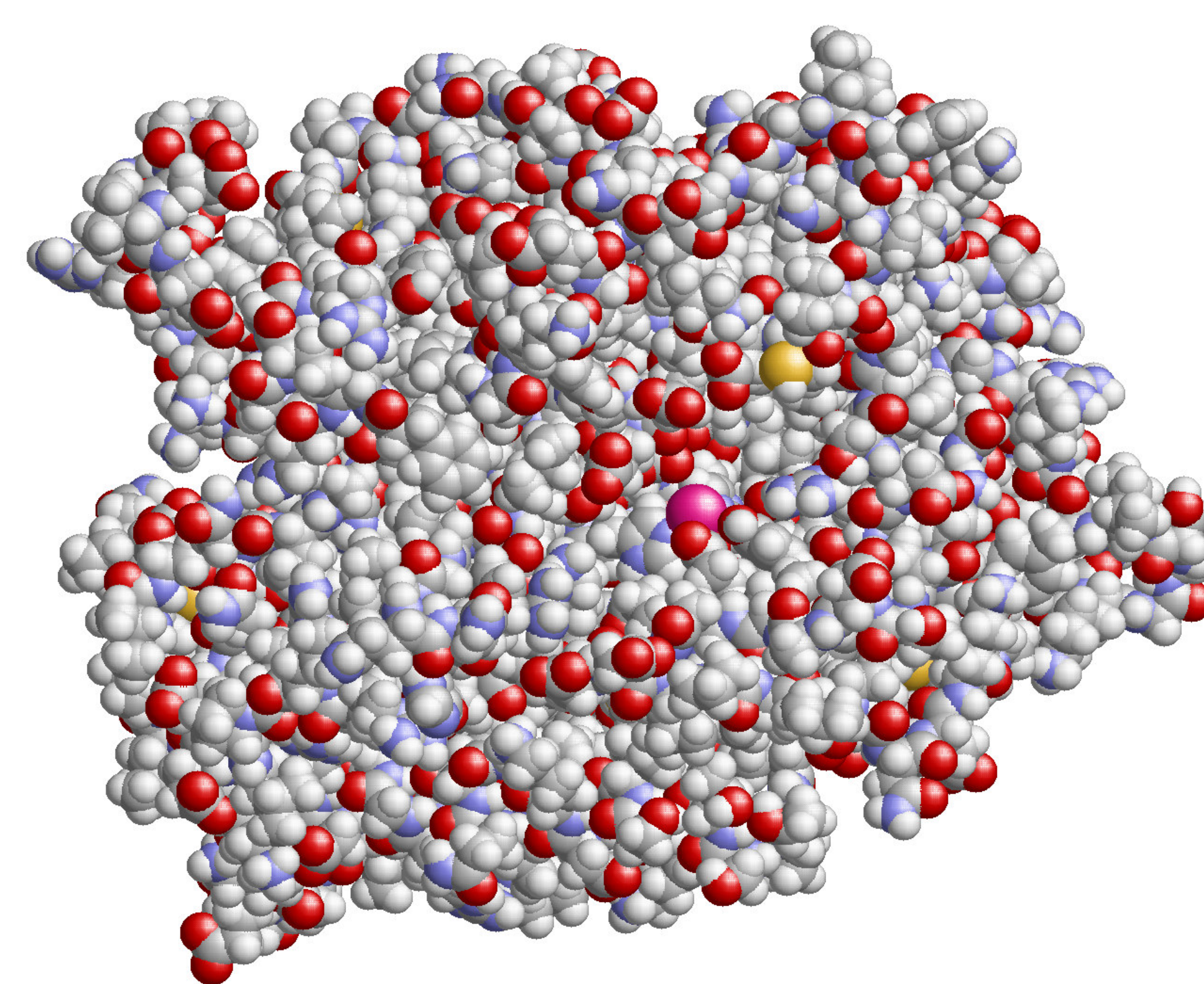
- Sarnoff Corporation (www.sarnoff.com) is conducting The Bug to Drug Program for the U.S. Government with the goal of developing an extremely rapid response to bio-warfare threats.
- A key element of this program is the design of small-molecule inhibitors to bio-warfare toxins.
- Sarnoff has developed a computational method to determine small molecules ligands given a protein target. (This technology has been licensed to Locus Pharmaceuticals, www.locuspharma.com, which is commercializing this capability for the pharmaceutical industry.)
- Over the past six months this has been applied to develop potential small molecule inhibitors for the botulism toxin.

Input to the method

- We used the protein structure for Botulinum neurotoxin type B with synaptobrevin-II bound, PDB code 1F83, by M. A. Hanson and R. C. Stevens [Nature Struct. Biol. 7, 687–692 (2002)]. Here the toxin is shown in green and the substrate in red and gray.



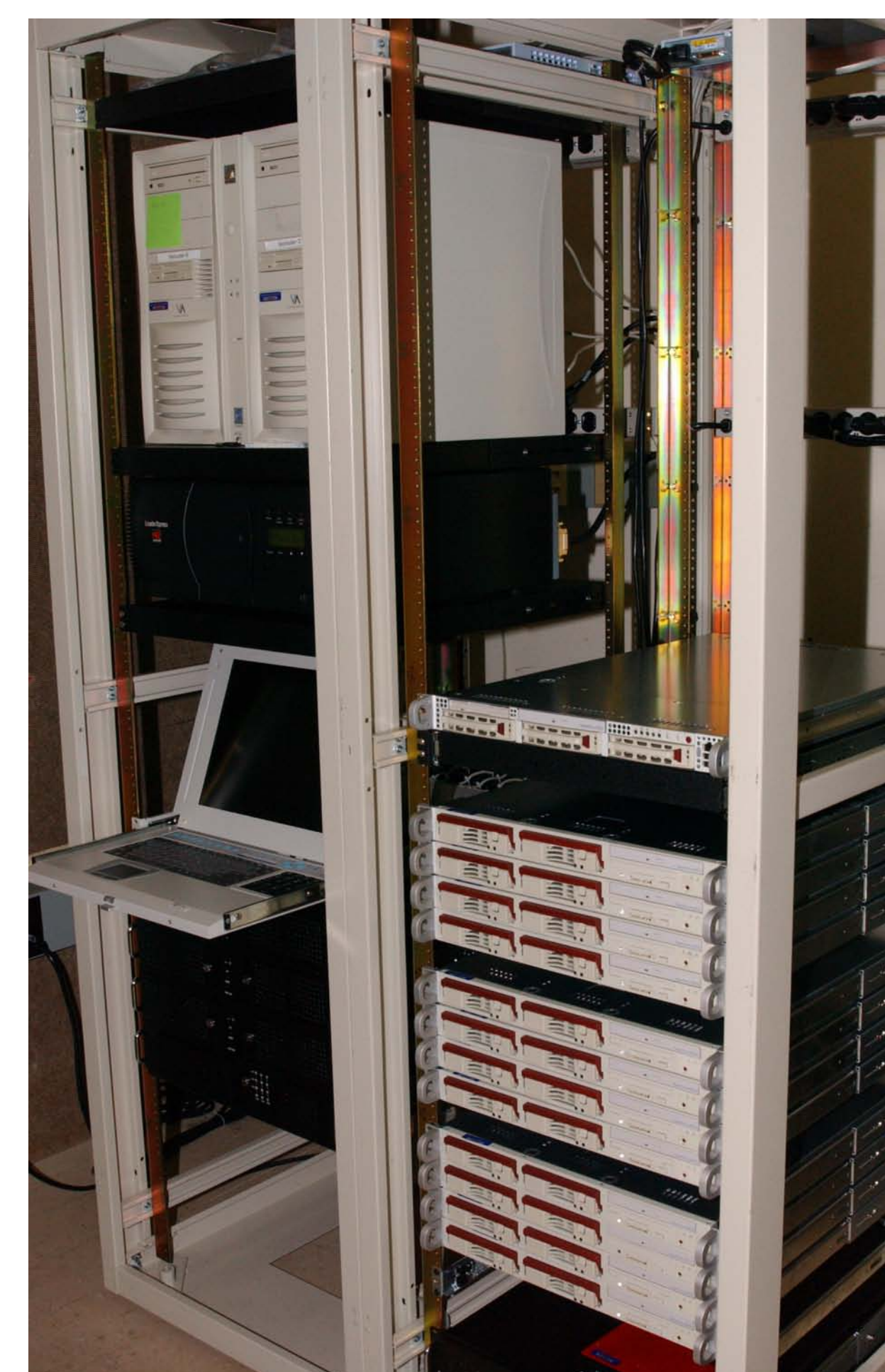
- We remove the synaptobrevin substrate prior to computation because we seek a small-molecule ligand which inhibits the binding of the toxin to the substrate. This shows the protein as used in the simulation.



- We also need a selection of small organic fragments with which to probe the protein. The structure of these can be determined *ab initio* by Gaussian.

The method

- A proprietary molecular simulation is performed on the the protein and each fragment. This is a computationally expensive operation. However, a PC cluster allows several years of computation to be carried out in approximately a week.

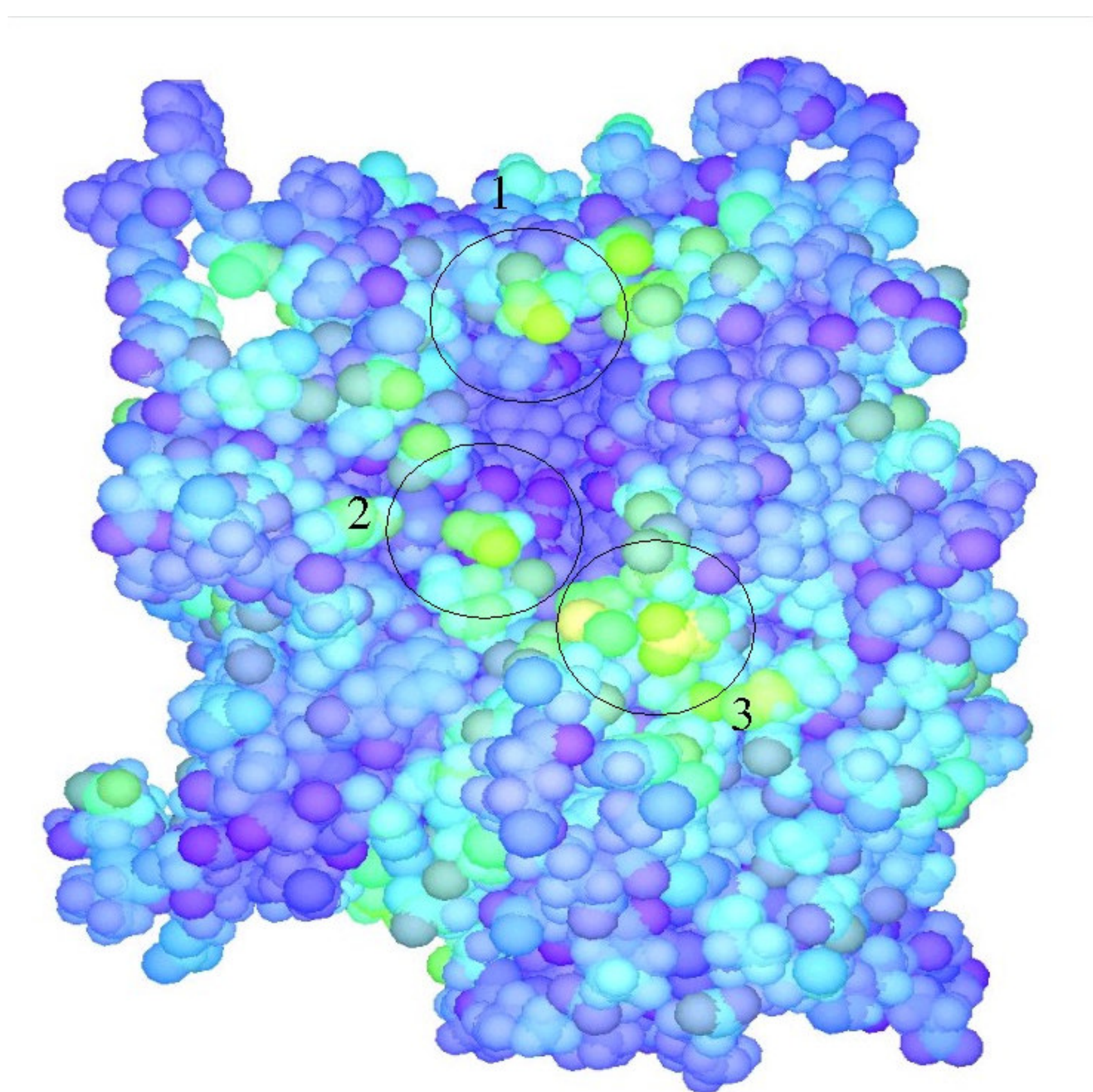


Sarnoff Biocluster specifications:

- 12 compute nodes: dual 1.26GHz Pentium III, 512MB RAM, 120GB disk.
- 21 compute nodes: dual 500MHz Pentium III, 256MB RAM, 9GB disk.
- 3 server nodes: AFS file servers, batch management.
- Software: Linux and our proprietary codes.

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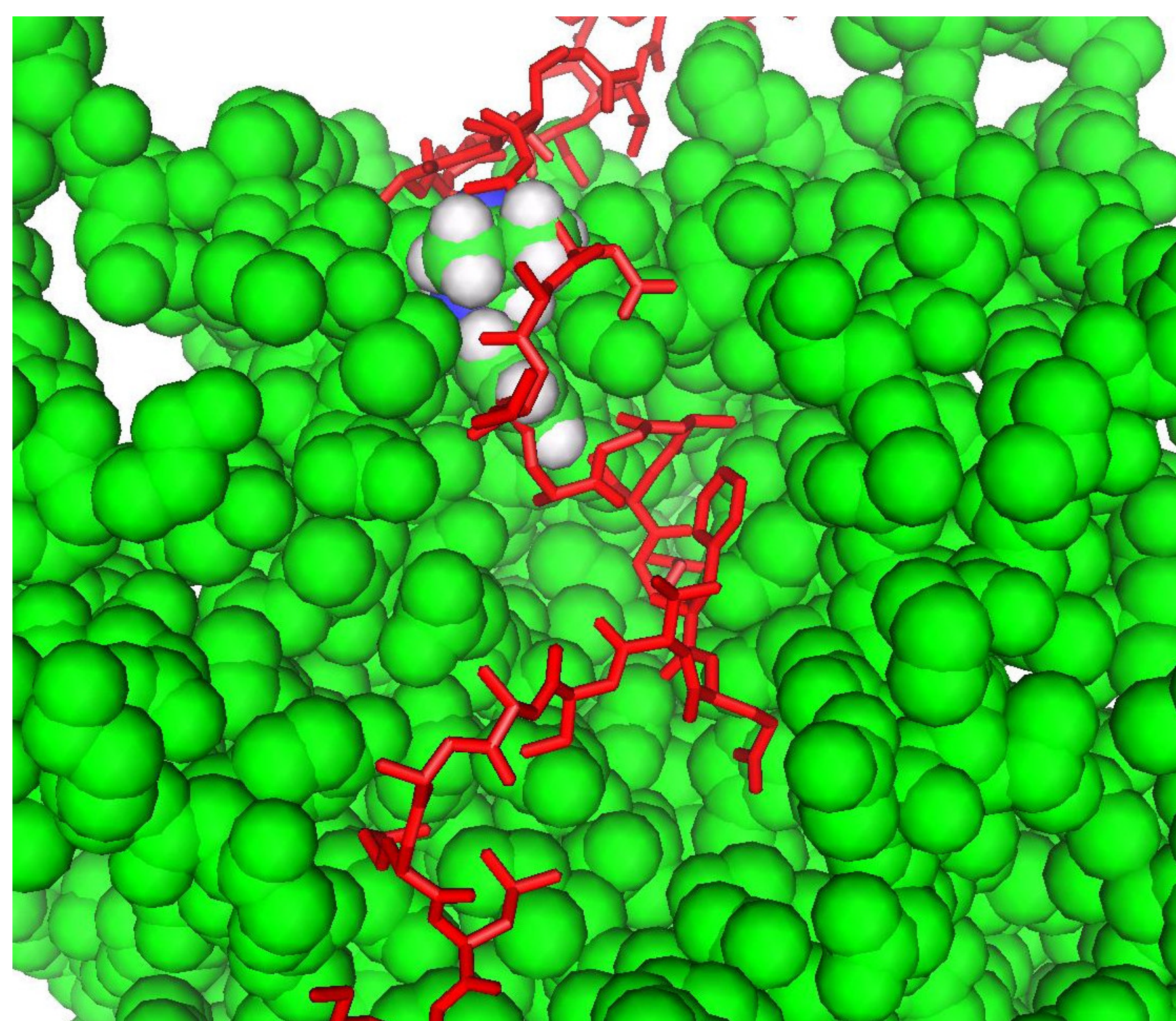
- The pattern of binding data is analyzed to find the relevant binding site on the protein. The binding site is further refined by the requirement that ligand disrupt the binding of the toxin to synaptobrevin.
- Binding site 1 is identified as the optimal site since this is directly under the SNARE secondary recognition sequence.



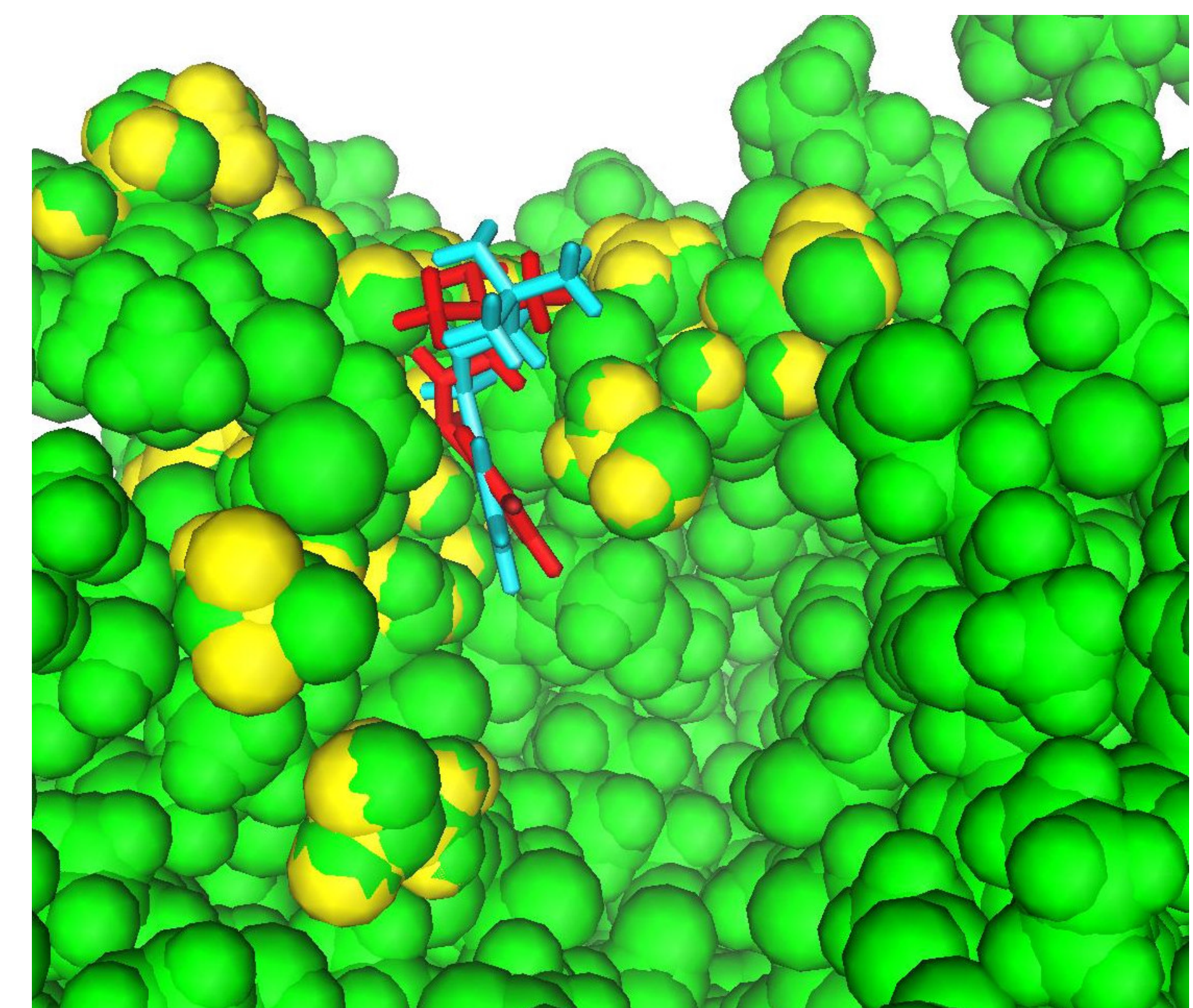
- The fragments are knitted together with another Sarnoff code to produce specific drug-like molecules which are predicted to bind to the protein with high affinity.

Results

- Approximately 50 candidate ligands were obtained by this process.
- Of these, 12 appear to bind strongly to the toxin.
- Molecular weights range from about 140 to 250, allowing the possibility to optimize the molecules for pharmacokinetic properties.
- A candidate inhibitor bound to BoNT (in green) with synaptobrevin superimposed as a red wire frame (from the 1F83 crystal structure).



- The same candidate small-molecule inhibitor following energy minimization. Red wire frame shows inhibitor in its designed position; cyan wire frame shows it in its energy minimized position; BoNT is shown in green (structure used for the design) and yellow (following energy minimization).



Advantages

- Given the protein target, the method can be used to predict drug leads in approximately 1–2 months.
- The method is inherently scalable. Locus Pharmaceuticals has a 1000-node (2000-processor) cluster on which it runs its versions of these codes. This allows a much richer set of fragments to be used.
- The resulting molecules are very specific to the protein binding site which minimizes the danger of unwanted side effects.
- Lead optimization can be performed using the computational data so that the high affinity is preserved during the optimization.

Future directions

- Synthesize and assay some of the predicted inhibitors.
- Apply to other potential bio-warfare toxins.
- Continue developing the software to improve its predictive capabilities.

Acknowledgments

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Additional information

For addition information, send E-mail to ckarney@sarnoff.com.