A hybrid method coupling normal mode analysis with projection to study structural flexibility of macromolecular assemblies

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Abstract
A hybrid approach that integrates Normal Mode Analysis (NMA) into a rigid-body, projection matching method has been developed to study conformational changes in macromolecular assemblies (large-scale structural rearrangements) [1, 2, 3]. The novelty of this approach is that it allows exploring all relevant intermediate conformations contrary to the existing methods that explore only the extreme conformations. It applies the normal modes on three-dimensional (3D) atomic coordinates of a macromolecular complex to determine the deformation of the 3D model giving the projection that matches best with the experimental two-dimensional (2D) image. The deformation parameters and the 2D-to-3D alignment parameters are determined simultaneously. This method is currently used to study the swelling mechanism of the Tomato Bubly Stunt Virus (TBVS) [5]. In this paper, we describe the NMA-based 3D-to-2D alignment method and show the results of its tests using synthetic data as well as preliminary results on experimental, cryo-electron microscopy data of the TBVS at different stages of swelling. In the future, this method will be tested on dynamic studies of other macromolecular complexes.

1. Type of structural flexibility to study: Large-scale conformational changes

Examples:
- Ribo/cage protein synthesis
- Ribosome RNA polymerase: transcription
- Virus maturation
- Compact, 13 A resolution
- Unprocessed and compact conformations
- Compact, 15 A resolution
- Unprocessed and compact conformations
- Cryo-electron microscopy [2002, 2004, 2006]: 2.5 A, 2.6 A, 3.7 A, 4.0 A, 4.4 A

2. Swelling of Tomato Bubly Stunt Virus (TBVS)

Simplified algorithms:
- Experimental image
- Orientation and translation
- Deformation
- Elastic deformation volume
- 3D atomic coordinates

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Details of the algorithms:
- 3D atomic coordinates
- Deformation
- Elastic deformation volume
- Orientation and translation
- Local search for orientation and translation
- Orientation and translation estimated on Stage 1 and adopted on Stage 2
- Orientation and translation estimated on Stage 1 and adopted on Stage 2
- Orientation and translation estimated on Stage 1 and adopted on Stage 2
- Orientation and translation estimated on Stage 1 and adopted on Stage 2
- Orientation and translation estimated on Stage 1 and adopted on Stage 2

3. Algorithm

Experimental image

3D atomic coordinates

Orientation and translation

Deformation

Elastic deformation volume

Details of the algorithms:

4. Tests with synthetic data computed using the TBVS atomic coordinates

Case 1: Images without noise and CTF influence

Normal mode analysis has been performed on the TBVS atomic coordinates (PDB code: 2TBV) and low-frequency normal modes were computed [2, 3]. The deformation induced by normal modes, along with deformation directions, do not break the symmetry of the structure (mode 9, 20, and 107). The mode 20 is responsible for scaling. Deformations along the remaining modes do not preserve the symmetry. We deformed the atomic coordinates with normal modes and magnified these deformations with a factor (3) to simulate large-scale deformations. With the following data, we computed three deformation amplifications: 0.500 along the mode 90 and 100 along the mode 107. The deformed structure was converted into a volume of size 256 x 256 x 256 voxels with a sampling step of 0.1 A, 1.1, 1.2 projections of the volumes were computed with random orientations determined by a set of three Euler angles uniformly distributed on the 3-sphere, and with uniformly distributed random translations (the standard deviation of noise applied on the coordinates of the outer sphere was 0.5). Deformation amplitudes along the three symmetrical modes (90.00, and 107). Euler angles and translations were estimated for each synthetic image using the NMA-based 3D-to-2D alignment method, and the estimated parameters were compared with their true values.

Results:
- Global angular distance: 4.30°
- Deformation amplitude RMS difference: 24 (mode 20), 15 (mode 90), 8.8 (mode 107)

Case 2: Images with simulated noise and CTF

The CTF and the noise were applied on the images computed for the case 1. The noise before the CTF (n) was modeled by a Gaussian white noise with the standard deviation. The noise after the CTF (m) was modeled by a five-pass modified Gaussian noise with the standard deviation generated at the input copy. The standard deviations were adjusted so that the ratio between the power of the signal and the power of the total noise was approximately equal to 0.5. The filter and the CTF were estimated simultaneously from a TBVS cryo-EM image for the following parameters of the electron microscope: accelerating voltage: 200 kV, defocus: -1.7 μm, spherical aberration: 0.5 mm. The CTF, the background spectrum filter, and the synthesized cryo-EM images were of size 256 x 256 voxels.

Results:
- Global angular distance: 4.0°
- Deformation amplitude RMS difference: 223 (mode 20), 240 (mode 90), 154 (mode 107)

5. Tests with experimental cryo-EM images of the TBVS: Preliminary results

Examples of the (noisy) cryo-EM images of the TBVS: Preliminary results

RMS, RM 310FF, 360K, C=0.5 nm

Deformation: 3.5 to 45 μm (microscopy): 1.7 to 3.2 μm (computed)

Magnification: 36000, Breyt carbon fiber, Low dose: 0.1 e/Å² Image size: 256 x 256 pixels. Sampling spacing: 1.6 A

Results of a test on a micrograph of the cryo-EM image (noisy 0.4 μm, computed defocus: 2.5 μm)

Deformation amplitude RMS difference: 420 (mode 20), 39 (mode 90), 35 (mode 107)

Results of a test on a micrograph of the TBVS cryo-EM image (noisy 0.4 μm, computed defocus: 2.5 μm)

Deformation amplitude RMS difference: 420 (mode 20), 39 (mode 90), 35 (mode 107)

Literature