Time-resolved serial crystallography to reveal protein structural changes

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Time-resolved serial crystallography (SX) is a technique used to investigate structural changes in proteins. These structural changes are induced within micrometer-sized protein crystals and are recorded as diffraction from X-ray pulses. Depending on the X-ray source and the nature of the reaction, time resolutions down to femtoseconds can be attained. This is especially useful for capturing transient or intermediate states, providing insights into the mechanisms and kinetics of biological reactions, including enzyme catalysis, conformational dynamics, and photoreactions.

ADVANTAGES:
- Ultrafast time resolution: enables real-time investigation of structural events.
- Atomic-level details: provides atomic-level structural information.
- Room-temperature data: preserves the natural state, dynamic behavior, and structural flexibility of the protein of interest.
- Sample flexibility: wide range of crystal samples, such as proteins, DNA, RNA, or complexes, can be used.
- Minimal perturbation: rapid probing with femtosecond X-ray pulses outruns radiation damage.
- Flexibility in experimental design: enables investigation of reactions triggered by various stimuli such as light, temperature, or substrate addition.
- Advanced X-ray sources: ultrashort X-ray pulses capture structural information with high signal-to-noise ratio.

CHALLENGES:
- Crystal requirements: requires microcrystals, which can be challenging to obtain for some biomolecules.
- Time resolution: achieving sub-millisecond timescales is limited to light-sensitive proteins.
- Non-photosensitive samples: it is difficult to engineer photo-triggerable chemical reactions, such as photosensitive caged substrates or pH changes, into non-photosensitive systems.
- Data complexity: the dataset is complex due to the large amount of diffraction data.
- Low hit rates: not all injected crystals will be hit by the X-ray pulses, resulting in a low hit rate.

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Thousands of diffraction patterns from small protein crystals are recorded at a free electron X-ray laser (XFEL) or a synchrotron source with a delay following an initial trigger (typically an optical laser). Protein crystals can be delivered by methods such as water or viscous jets, microfluidic devices, tape-drives, or as fixed targets. Data analysis is then used to recover the time-dependent structural information.

Declaration of interests
No interests are declared.

Literature

Sample heterogeneity: biological samples can exhibit heterogeneity, resulting in a mixture of different conformations or intermediates, making data analysis more challenging.