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RADIATION DAMAGE DICTATES THE PRACTICAL DETAILS OF HOW SINGLE-PARTICLE

BIOLOGICAL CRYO-EM IS DONE

CRYO-EM SPECIMENS ARE VERY SENSITIVE TO RADIATION DAMAGE

- **The resolution of visible spots in the FFTs of images of a thin catalase crystal decreases as the accumulated electron exposure is increased**
- **If the signal did not fade away with exposure, then longer exposures would produce better signal-to-noise**

• High-resolution feature fade much faster than lower res^{n}

– The optimal exposure thus depends upon resolution

Baker, Smith, Bueler & Rubinstein.(2010) J. Struct. Biol. 169:431-437.

GRAPHENE CAN TOLERATE EXPOSURES >> 10^4 e/Å2; WHY IS THAT NOT TRUE FOR BIOLOGICAL MACROMOLECULES?

• Again, high-resolution features of protein structure are destroyed after exposures of ~10 e/Å^2 at liq N2 temperature, and much faster at room temp

– Empirically determined – Low-resolution features survive 5-10 times longer See: Taylor & Glaeser

(1976)Journal of

Ultrastructure Research 55, 448-456 Glaeser &Taylor. 1978. Radiation-Damage Relative to Transmission Electron-Microscopy of Biological Specimens at Low-Temperature - Review. Journal of Microscopy-Oxford 112:127-138.

- **FOR THOSE OF YOU WHO KNOW ABOUT CHEMICAL BONDS:**

– Changes in bond order when a single electron is removed (ionized) from a large, conjugated-electron system vs from a saturated-bond system – Only knock-on damage & Auger events limit the exposures for graphene – Whereas ionization damage limits the "safe" exposure for proteins

INELASTIC ELECTRON SCATTERING IS WHAT CAUSES STRUCTURAL DAMAGE

- **The majority of inelastic scattering events deposit about 25 eV per event – These primary**

events involve collective excitation of all electrons within a small neighborhood (plasmon excitations)

– These initial excitations rapidly decay into one or more ionization events plus localalized heat

- **Nearly every such event produces, on average, one break in any type of organic (aliphatic) target-molecule**
- **Exposures of ~10 e/Å^2 deposit ~10^{23}eV/g or ~5x10^9 rads**

– **Energy densities this high are found in few locations on earth other than a cryo-EM specimen**

Figure taken from Aronova, M. A., & Leapman, R. D. (2012). Development of electron energy loss Spectroscopy in the biological sciences. *MRS bulletin / Materials Research Society, 37*(1), 53-62. doi: 10.1557/mrs.2011.329

RADIOLYSIS MAKES IT IMPOSSIBLE TO RECORD INDIVIDUAL, HIGH-RESOLUTION IMAGES

IMAGES TAKEN WITH "SAFE" EXPOSURES ARE TOO NOISY

- **High-resolution features become buried in the noise as the exposures becomes lower and lower**

– i.e. the random spatial distribution of electrons becomes more and more noticeable as the events become more and more sparse

A version of an empirical inequality established by Albert Rose Rose, A. 1973. Vision: human and electronic. Plenum Press, New York

From: Glaeser, R. M. 2008. Retrospective: Radiation damage and its associated "Information Limitations". Journal of Structural Biology

163:271-276.

Images from many particles must thus be averaged in order to see high-resolution detail, even for a single pose

These images are sometimes called “2-D class averages”

IT IS EVEN FURTHER IMPOSSIBLE TO RECORD HIGH-RESOLUTION 3-D DENSITY MAPS OF A SINGLE PARTICLE

- Instead, data must be merged from many more particles, in different poses, in order to get a well-defined, 3-D density map – There are many possible ways to merge data, most of them not even using class

averages

- Many 10's-of-thousands of particles are typically used to ultimately produce a 3-D map that is not noise-limited
  - Although as few as 2000 would be enough if images were perfect

3-D maps are "interpreted" by building a chemical structure that fits into the map in every detail

ESTIMATES OF WHAT IS POSSIBLE WHEN DATA ARE MERGED: WHETHER SPA OR SUB-TOMO AVERAGING

- Physics allows EM to get 3 Å structures by merging data from ~12,000 particles,

which must be

~40 kDa or larger –
Henderson 1995

(Quart. Rev. Biophys.

28:171-193)

- More optimistic

estimate: ~1400

particles, MW > 17

kDa

- Glaeser 1999 (J.

Struct. Biol. 128:3-14)

- An even slightly

more optimistic

estimate has been

made!

- Rosenthal &
Henderson 2003 (J.

Mol. Biol.

333:721-745)

- Cryo-EM

structures have

been obtained of

the iconic, 64 kDa

hemoglobin

molecule

- But only after merging data from >>10k particles
- And attempts to get structures of <50 kDa particles have so far failed

Unphysical values are required for the density of the particle vs the surrounding water

Divide by 100 to get e/Å ^{2}

Based on the Rose equation for distinguishing and object from its surronding area

Glaeser, R. M., and R. J. Hall. 2011. Reaching the Information Limit in Cryo-EM of Biological Macromolecules: Experimental Aspects. Biophysical Journal

100:2331-2337.

ESTIMATES OF WHAT IS POSSIBLE FOR SINGLE PARTICLES: TOMOGRAPHY RADIOLYSIS ALSO CAUSES BEAM-INDUCED MOTION

- A current model (mine, anyway) is that mechanical stress within the thin-foil sample changes as it is being irradiated
  - The thin foil responds by flexing and

bending – So-called ‘drum-head” or “doming” motion

- If the sample is locally tilted by even a few degrees, this Z-motion translates to a signifcant horizontal component

Example of MotionCorr2 tracking of image (and sub-region) motion during a movie

Zheng, Palovcak, Armache Verba, Cheng, & Agard. 2017. Nature Methods 14:331-332.

MORE ABOUT THE MODEL THAT SPECIMEN MOTION IS ATTRIBUTED TO “DOMING”

• The model "drumhead" of specimen or motion "doming" – Vertical may usually changes be ignored in position • The enough depth to of not field worry is great - usually – But may just the specimen the a be small significant, horizontal tilt of the component even plane for of • Motion first (Burst few phase) is e/Å^{2}greatest of exposure during the – Slows exposure significantly continues as the • Charging plays no significant does occur, role but it • HYPOTHESIS REPEATED:

– Stress unequal is created thermal during contration vitrification of components due to – This stress is released during the first, burst phase – But irradiation new stress

continues
continues to be generated as
Russo, C. J., and L. A. Passmore. 2014. Ultrastable gold substrates for electron cryomicroscopy. Science 346:1377-1380.

DOSE-FRACTIONATED "MOVIE-MODE" IMAGES ARE A MAJOR ADVANCE, BUT STILL FAR FROM PERFECT

AS SAID PREVIOUSLY, RADIATION DAMAGE IS CAUSED ALMOST COMPLETELY BY INELEASTIC SCATTERING OF ELECTRONS

- We cannot pretend that electrons never lose energy when passing through thin samples
- Inelastic scattering occurs, in fact, about three times more frequently than elastic scattering!
- But knock-on damage can be ignored completely for biological molecues because it happens so rarely – Although knock-on is the main limitation for imaging metals, minerals, and most other hard materials

Most electrons are never scattered

Some, however, are inelastically scattered
inelastically scattered Some, however, are
Some, however, are inelastically scattered

RUPTURE OF CHEMICAL BONDS IS ~IRREVERSIBLE

- CRYO-TEMP DOES NOT MITIGATE THE RATE AT WHICH INTIAL (PRIMARY) DAMAGE OCCURS – This is just physics
- Instead, reactive species go on to attack neighbors if they can, and thus do secondary damage – This is chemistry
- CRYO-TEMPERATURES HELPS BECAUSE PRODUCTS OF RADIOLYSIS

ARE “CAGED” – Secondary damage is greatly reduced or even eliminated

THIS FIGURE IS JUST EYE-CANDY It does not really match the points that I am making

FURTHER NOTE THAT: 1. Radiolysis reactions follow many possible paths, few of which lead back to the parent 2. Covalent bond lengths become van der Waals contacts, and the daughter fragments no longer fit into the parent cavity

THE RESULTING MISFIT GENERATES STRESS 3. The initial recoil during bond rupture embeds fragments far enough into the surrounding matrix that broken bonds cannot reform

WATCHING MOLECULES – AND EVEN CELLS – FUNCTION IN LIQUID WATER IS A VAIN HOPE

Glaeser, R. M. 2016. Chapter Two - Specimen Behavior in the Electron Beam. In Methods in Enzymology. R. A. Crowther, editor. Academic Press. 19-50

SUMMARY

- Biological macromolecules are sensitive to ionizing radiation

– Electrons are strongly ionizing radiation

- This limits the electron exposure that can be used to record images

– Merging data from 10's of thousands of particles overcomes the problem that the noise-level is otherwise to high

- Radiolysis also releases

pre-existing mechanical stress, and generates additional, new stress

– Movie-mode data acquisition largely, but not completely recovers detail that is otherwise lost due to this motion