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Adventures in Phasing: Flavivirus NS1 solved by low resolution native S-SAD

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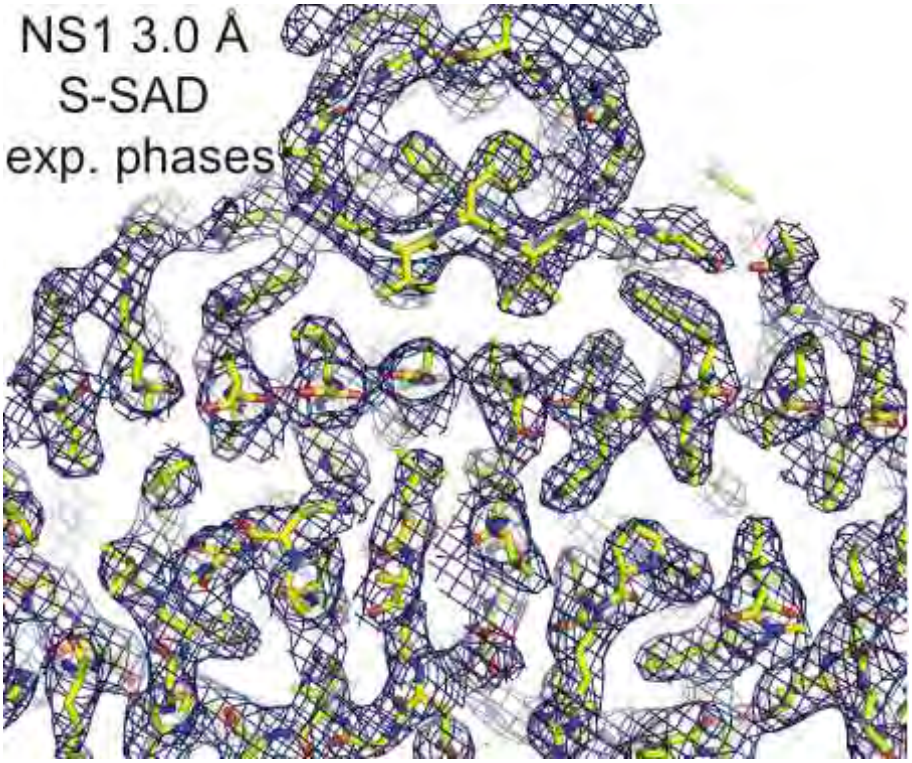
We used native sulfur-SAD phasing to determine a novel structure of significant health importance, the flaviviruses non-structural protein 1 (NS1). Flaviviruses cause many diseases including dengue and West Nile fever. NS1 functions in both genome replication and immune system evasion. Full length, glycosylated NS1 was expressed in insect cells and crystallized. Due to difficulties with protein expression SeMet phasing was not practical, and we embarked on an effort to use sulfur-SAD phasing. To optimize the probability of observing the estimated 1.5% S-anomalous signal, we used inverse beam protocol at 7.1 keV with 5° wedges and 0.5° oscillations. Crystals typically diffracted to ~3.0 Å. Pairwise comparisons of anomalous correlation coefficient (AnomCC) and Rmerge in the highest intensity (low resolution) bins were used to decide which crystals to include in the final data set. Crystals which when compared to other crystals consistently had AnomCC < 0, or Rmerge > 7.5% in the low resolution bin were excluded. Data from 18 of 28 crystals were combined to generate a data set with ~100 fold anomalous multiplicity. The final data set was of high quality by I/σI and CC1/2 metrics with a positive AnomCC to ~5.4 Å. Sulfur sites were found with SHELXD using data to 5.2 Å. Phases calculated to 4.5 Å by SHELXE and extended to 3.0 Å with DM were of sufficient quality to autobuild ~75% of the final model. The NS1 structure was complete with the exception of one 20-residue internal loop, and glycosylation was observed at expected sites. Although Rmerge values were high for the combined data (30% overall, 9% low-res, 900% hi-res), model building and refinement proceeded smoothly, supporting the notion that Rmerge is a poor indicator of data quality for purposes of refinement and may also be a poor indicator for purposes of phasing. The effects of data multiplicity and resolution on S-site determination, phase calculation and phase extension were investigated.

[1] *D. Akey, W. Brown, S. Dutta, et al., Science, 2014, [DOI:10.1126/science.1247749]*



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