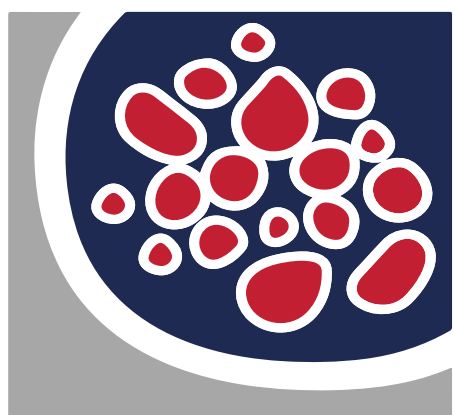


Exploring processing of baculovirus P10 protein using next-gen protein sequencing

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Challenge: OET observed that the 10 kDa baculovirus P10 protein band was replaced by an 8 kDa band on SDS-PAGE gels during infection. They needed to confirm whether the smaller protein originated from P10 and if so identify the location of proteolytic cleavage.



Innovation: OET combined SDS-PAGE and Western blotting with Quantum-Si single-molecule protein sequencing to characterize the two protein bands. This approach enabled direct identification and comparison of P10-derived peptides throughout infection.



SMPS Integration: SMPS confirmed that the 8 kDa band was a truncated form of P10 and showed that cleavage did not seem to occur at the predicted amino acid 59 site. Loss of the C-terminal SQA peptide instead implicated a cleavage region downstream of amino acid 72.



Impact: The results replaced the original cleavage hypothesis with a new evidence-based mechanism for C-terminal P10 cleavage. These insights advance understanding of baculovirus biology and support the development of improved expression systems

INTRODUCTION

Oxford Expression Technologies (OET) is a UK-based biotechnology company specializing in recombinant protein and virus-like particle production. With 25 years of experience in protein expression, they combine established technologies with specialist scientific expertise to help their customers overcome complex expression and production challenges.

Their expertise spans insect, mammalian, and bacterial expression systems, supported by their proprietary *flashBAC™* baculovirus expression platform, specialist cell lines, and a comprehensive range of protein expression and production services.

From gene design and recombinant virus generation through expression optimization, purification, scale-up, and protein sequencing, they provide flexible, end-to-end solutions tailored to each project. OET work closely with their customers throughout the process, providing technical expertise, practical support and a collaborative approach to help turn challenging protein expression projects into reliable results.

POTENTIAL USE CASE FOR SMPS (SINGLE-MOLECULE PROTEIN SEQUENCING)

Quantum-Si single-molecule protein sequencing can be used to characterize recombinant protein products by confirming protein identity and assessing sample composition at the individual-molecule level. The technology identifies protein-derived peptides and provides detailed amino acid sequences aligned to a reference sequence. This allows detection of different proteoforms. It also allows samples to be examined for the presence of host cell proteins using an inference workflow to examine the data obtained against a proteome sized database.

These capabilities provide practical use cases for verifying that the intended recombinant protein has been expressed, identifying heterogeneity or processing, and comparing product characteristics across difference batches or conditions.

Compared with conventional mass spectrometry, Quantum-Si provides a complementary, direct single-molecule view of individual peptide molecules without relying on ionization or peptide-spectrum matching. Quantum-Si can offer a simple, accessible, and targeted workflow for confirming recombinant protein identity, resolving molecular heterogeneity and comparing the relative abundance of specific peptides across samples.

BACKGROUND ON BACULOVIRUS P10

The **P10 protein** is a highly abundant baculovirus structural protein encoded by the **p10 gene** of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV). It is expressed during the late stages of infection and is associated with the formation of fibrous structures and the reorganization of the infected cell. P10 is also linked to cytoskeletal changes, nuclear disruption, and the formation of cellular structures that accompany baculovirus infection. Despite its high abundance, the precise molecular functions of P10 are not fully understood, making it an interesting target for studying baculovirus biology and virus-host interactions.

OET combines fundamental baculovirus research with applied biotechnology to continually improve expression technologies. Our work on the AcMNPV P10 protein provides insights into viral protein expression, processing and virus-host interactions, helping us better understand baculovirus biology and inform the development of next-generation baculovirus-based expression systems.

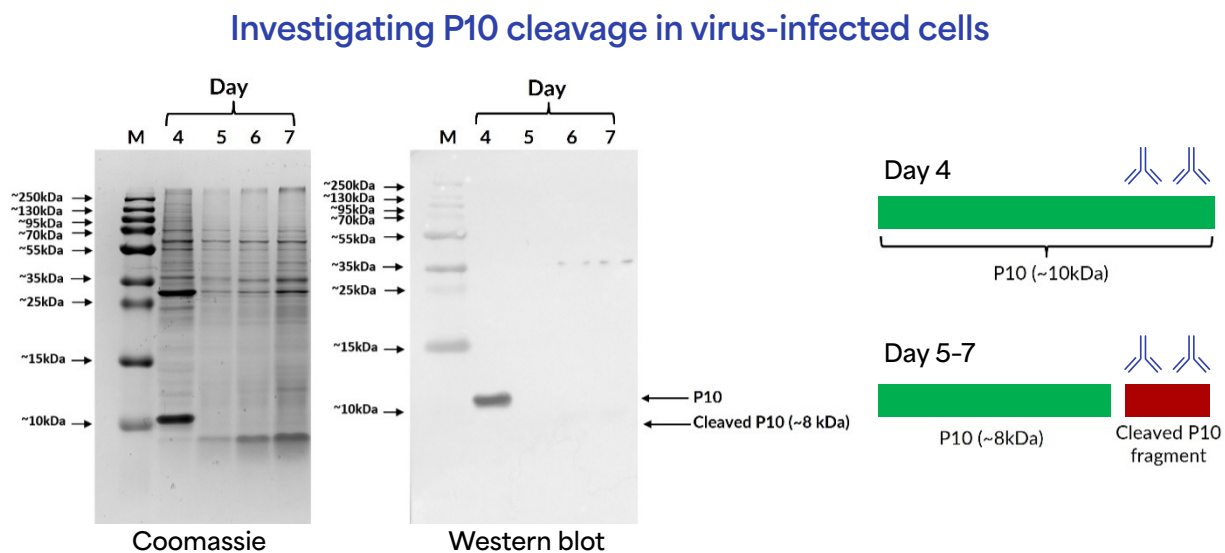
Understanding the expression, processing and function of P10 can therefore help advance our knowledge of baculovirus infection and inform the development and optimization of baculovirus-based expression systems.

While P10 migrates at the expected 10 kDa on an SDS-PAGE gel, OET have noticed an additional 8kDa band appears on SDS-PAGE gels of proteins from infected cells four days after

infection (see figure below), which coincides with the disappearance of the 10 kDa band. They suspected that this 8 kDa protein was a truncated form of P10 that had been subjected to proteolysis, and wished to confirm the location of the cleavage site and whether the theorized location (AA59) was the site cleaved by viral cathepsin.

RESULTS AND DISCUSSION

Coomassie and Western blot data:

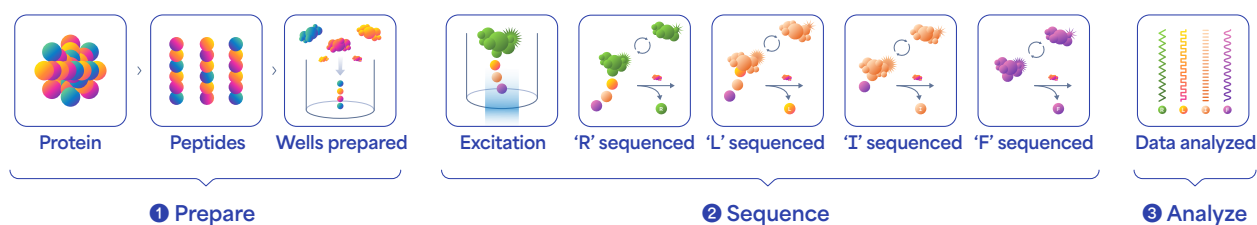


Studies using monoclonal antibodies have demonstrated that P10 contains antibody-recognized regions. These were mapped using recombinant fragments of the P10 protein and were found to be located at the C-terminal of the protein. It is notable that the 8 kDa band, which is hypothesized to be cleaved P10, is not detected by the anti P10 antibodies. An alternative means is required to determine if this 8 kDa band is P10 related – the 10 kDa band seen on day 4 and the 8 kDa band seen on days 6,7 were excised and subjected to SMPS.

APPLICATION OF SMPS

Single-molecule protein sequencing for Quantum-Si uses a novel detection mechanism: recognizers detect specific amino acid types with distinct pulse durations, and the addition of aminopeptidases enables sequential exposure of downstream amino acids – generating characteristic kinetic signatures called recognition segments (RS). These kinetic signatures uniquely identify proteins and proteoforms, and analysis software automatically

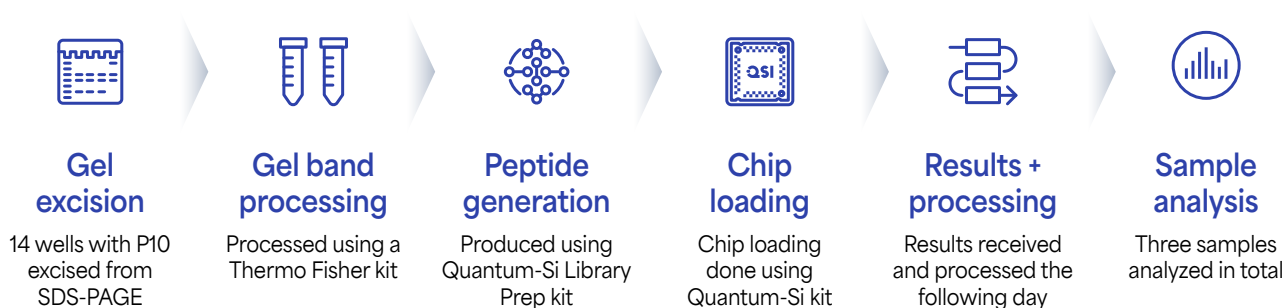
delivers single-molecule-level information without the need for bioinformatics expertise. It uses a bottom-up workflow where Lys-C digested peptides are immobilized onto a chip where the sequencing reaction takes place using the Platinum® Pro instrument. The technology combines versatility with accessibility and was deemed ideal for further investigation of the P10 protein.

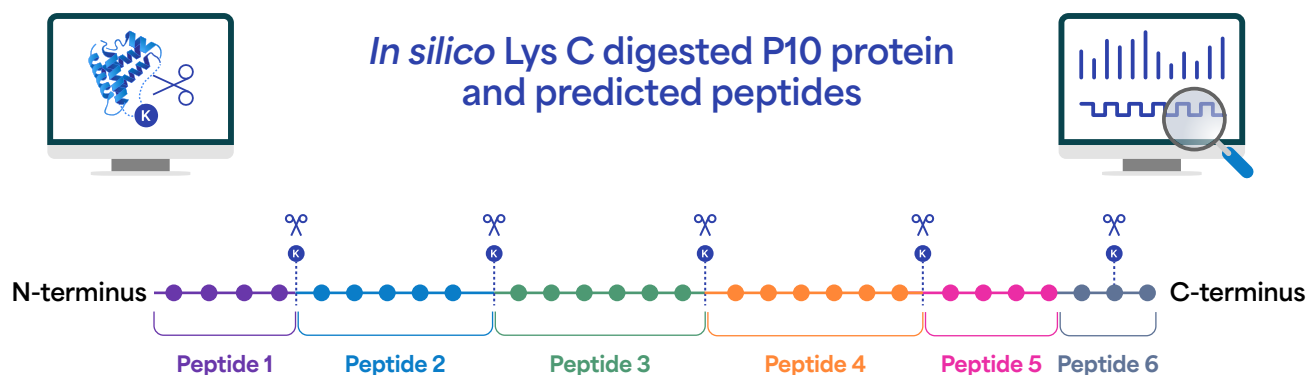


Recognizers bind to individual N-terminal amino acids



P10 proteomics workflow





P10 peptides

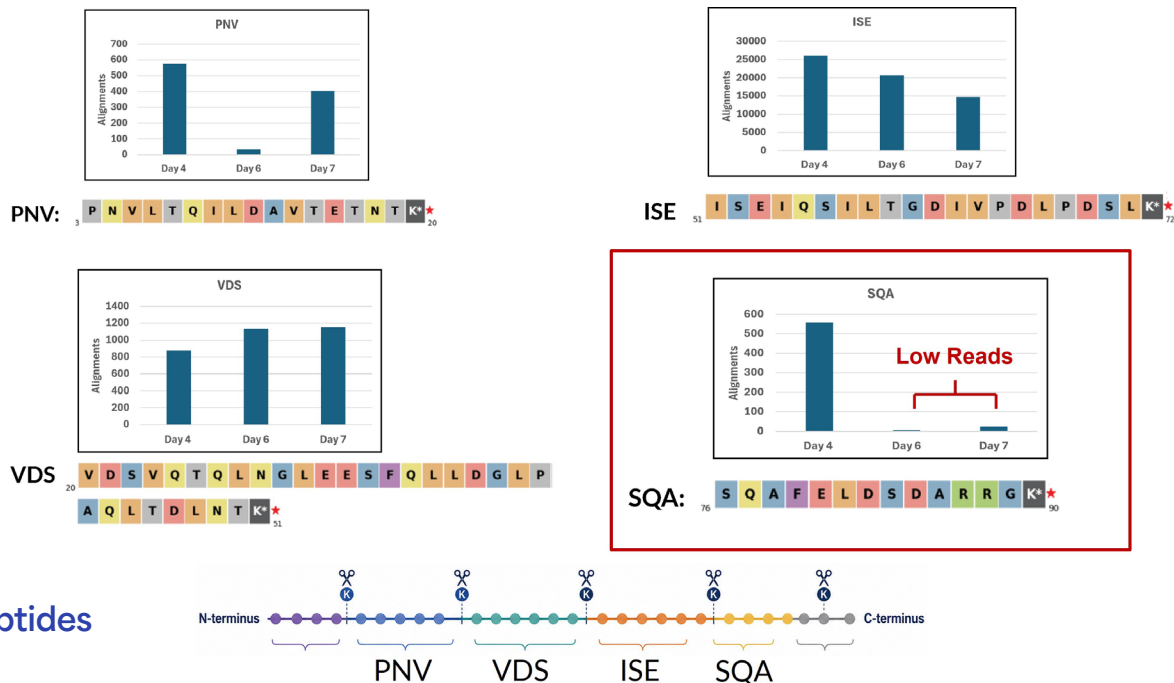


P10 will digest into eight peptides, of which four (PNV, ISE, VDS and SQA) were of sufficient length and had suitable amino acid compositions (recognized amino acids) that made them expected to be visible using the assay. The smaller peptides MSK, PK, LK and RSSK were not expected to be sequenced in the *in silico* analysis. While the next-generation Quantum-Si instrument, Proteus, will have complete AA coverage, Platinum is ideally suited to what was done in this project.

The analysis software workflow outputs detailed sequence data of the peptides detected, together with the number of times that peptide was detected on the chip and the false discovery rate associated with that peptide. The number of alignments and FDR obtained for each peptide were examined.

SEQUENCING RESULTS

Peptide alignment reveals a loss of C-terminal peptides



When the number of alignments and FDRs relating to each peptide were examined, some noticeable trends were observed.

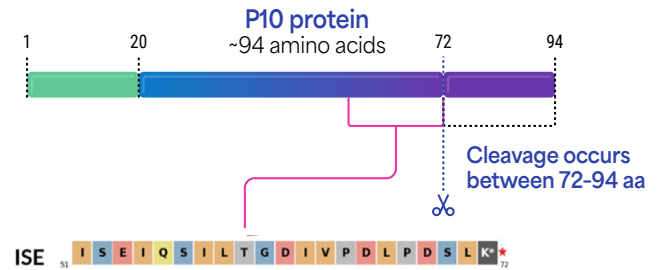
- SQA: The most dramatic result was seen with the C-terminal SQA peptide which was strongly detected in the 10 kDa band from day 4 but did not generate significant alignments (FDRs were high) on days 6,7 when isolated from the 8 kDa band, suggesting it is part of the missing fragment.
- The ISE peptide demonstrates a gradual decline in alignment numbers over the course of the infection, although FDRs were significant in all cases. It is notable that the ISE peptide contains the currently hypothesized cleavage site at amino acid 59. The presence of sequence data beyond this point in the peptide suggests that the cleavage did not, in fact, occur at this point.
- VDS is strongly detected throughout, with many alignments and strong FDRs, confirming that there was not total protein loss during the course of infection, and that the 8 kDa fragment is indeed a truncation product of P10
- PNV (at the N-terminal) gave a pattern where read numbers were high on day 4, fell back on day 6, and then partially recovered on day 7. FDRs reflected this pattern too. This represents an intriguing avenue for future investigation.

NEW THEORY BASED ON QUANTITATIVE RESULTS

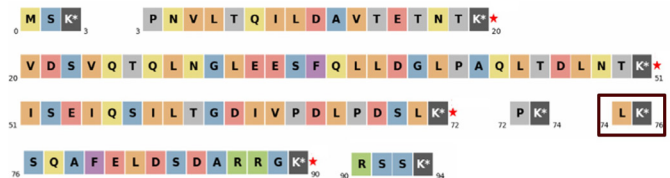
- Previous studies suggested P10 may be cleaved by viral cathepsin (V-CATH) at AA 59
- Quantum-Si results indicate that cleavage appears to be isolated to the C-terminus
- Further biometric analysis within the C-terminus identified several putative cathepsin cleavage sites
- AA 76 represents a very strong candidate site due to the presence of both a Leu (L, position 2) and Lys (K, position 1)
- Cleavage at AA 76 would generate an ~8 kDa fragment (and +2 kDa) at the C-terminal, consistent with the SDS-PAGE results

P10 cleavage site mapping

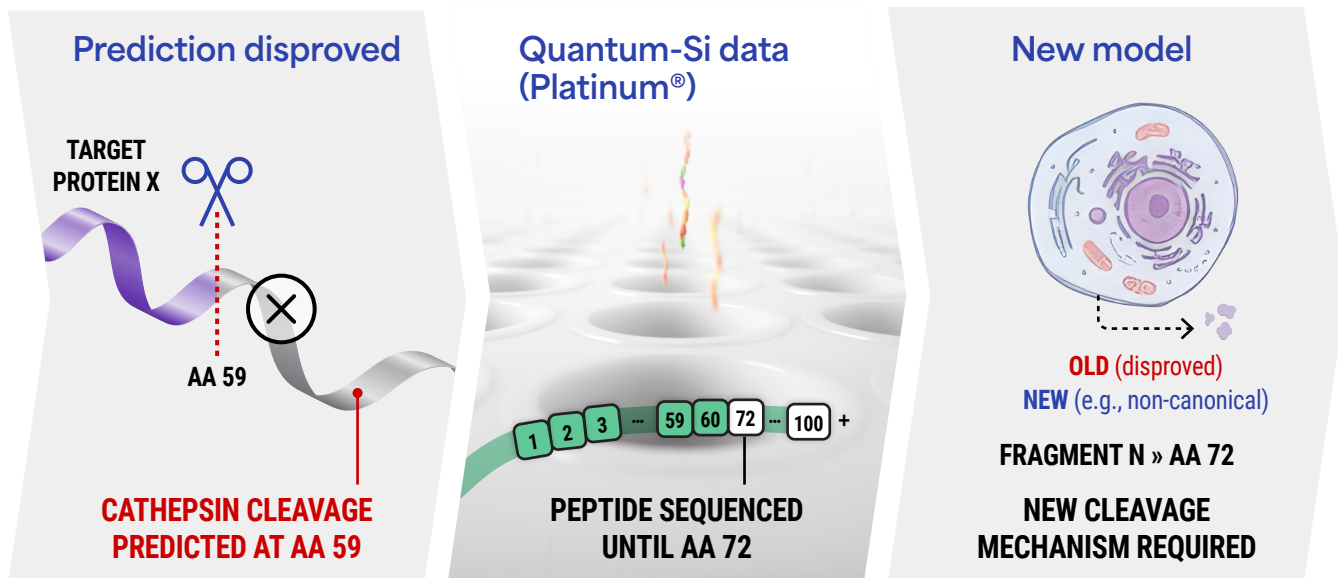
Based on Quantum-Si sequencing data



P10 peptides



NEW MODEL/THEORY



V-cathepsin may cleave P10 at several sites, and identifying the relevant site would help clarify its mechanism of action. Initial bioinformatic analysis predicted cleavage at amino acid 59, but detection of the ISE peptide indicates

that this is not the only cleavage site. Attention has therefore shifted to candidate sites in the C-terminal region, downstream of amino acid 72, which are consistent with the loss of the SQA peptide and the retention of ISE.

Bioinformatic analysis conducted by OET suggests that LKPKLKT (aa ~68-74) is probably the strongest candidate site because it contains multiple Lys residues, lies within the flexible central region of the protein, and is likely to be solvent exposed. While similar Lys-rich motifs are recognized by cathepsin L-like proteases, their specificity is broad rather than sequence-specific.

Identifying the cleavage site would strengthen a model in which a P10 cage initially forms around the nucleus, preventing premature release of polyhedron-encased occlusion-derived virions (ODVs). At approximately 72-96 hours post-infection, V-cathepsin may disrupt this cage by cleaving P10, releasing the ODVs in coordination with nuclear lysis.

Similar Lys-rich motifs are recognized by cathepsin L-like proteases, although specificity is broad rather than sequence-specific.

CONCLUDING REMARKS

The data presented here show the ability of Quantum Si's single-molecule sequencing technology to add additional data richness to existing SDS-PAGE and Western blot data, which complements OET's commitment to gaining a deeper understanding of the baculovirus expression system, allowing them to continue to innovate and further enhance their capabilities.