Proteomic sequencing and resurrection of a monoclonal antibody

DIGITAL PROTEOMICS

LakePharma

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Motivation

While hybridoma sequencing has become a routine part of drug development workflows, there are few options for antibody primary sequence determination when the source hybridoma is unavailable. Mass spectrometry offers a high-throughput method for interrogating the proteome. At Digital Proteomics, we've developed Valens, which combines an antibody-specific mass spectrometry protocol with sophisticated informatics to address the challenge of proteomic sequencing of antibodies.

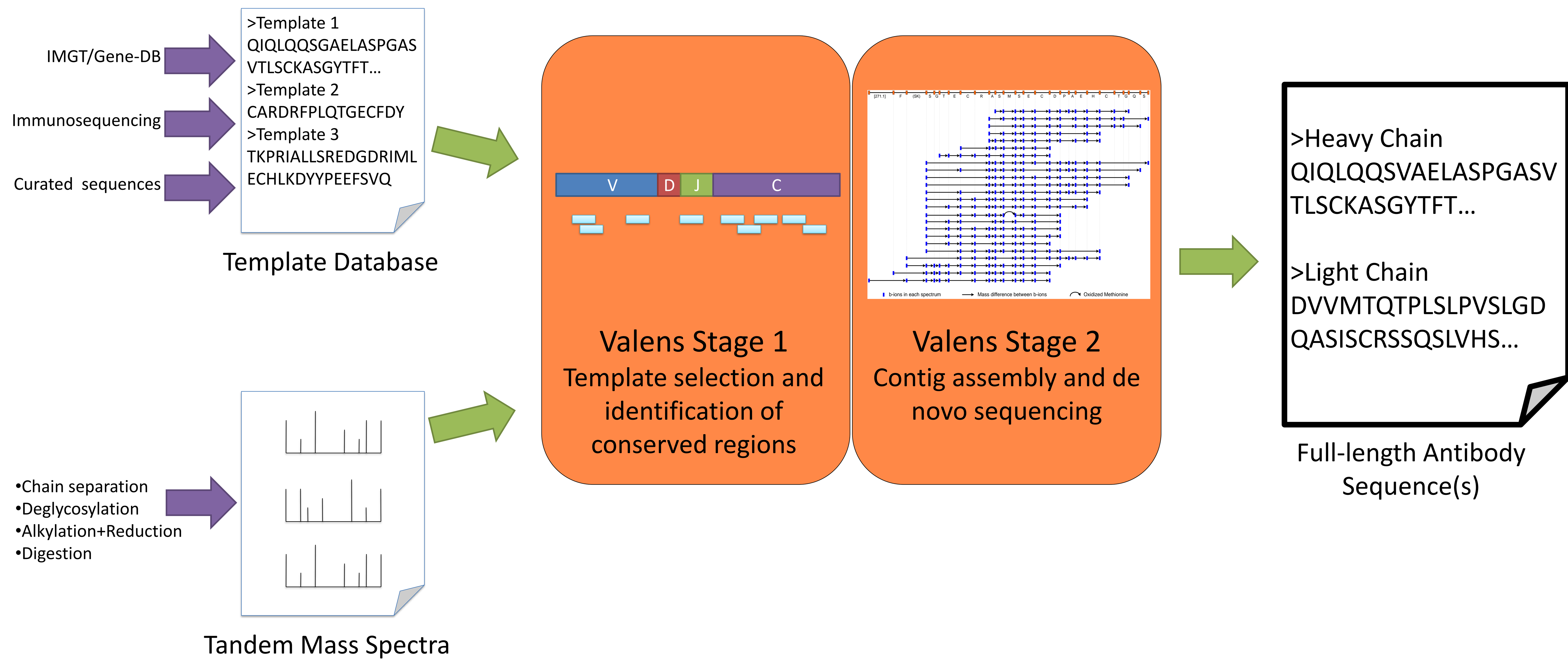
FLAG-tag is a polypeptide tag that can be conjugated to a protein, enabling quantitative investigation of a protein(s) of interest. The anti-FLAG M2 antibody targets FLAG-tag and is a crucial component of the proteomic assays. A preliminary protein sequence of an anti-FLAG tag antibody was recently published for the purposes of elucidating the crystal structure of the antibody in complex with trans-membrane proteins (Roosild *et al*, 2006). Protein sequencing was performed using Edman degradation, however, this method only revealed the first 40 amino acids and several gaps persisted, including in the CDR3. Recently, Digital Proteomics LLC developed a monoclonal antibody sequencing technology, Valens, which employs mass spectrometry to determine the full antibody sequence. In collaboration with LakePharma, Inc., we determined the antibody sequence for the anti-FLAG M2 antibody.

Protein Digestion

A single MS run (~4500 spectra) of a single enzyme only covers about 50% of the antibody sequence. By using our recommended five enzymes (trypsin, chymotrypsin, pepsin, elastase, and AspN), we routinely achieve over 99% coverage.

QIQLQQSGAELASPGASVKLSCKASGYTFTDHIMNWVKKRPGQGLEWIGRIYPVKKETNY

Proteomic Sequencing using Valens



Final Sequences

Heavy Chain

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Valens     EVQLQQSAAELARPGASVKMSCKASGYXFTXYXIHWVKQRPGQGLEWIGYIXPXXGXXXYNQNFKDETTLTADPSSS
Published  EVQLQQSGGEL?KPGASVKMSCK?SGYTFT?Y?IHW?KQ?-G?GLEWIGYI?P??G??-YN??FKGK?TL??DKSSS

Valens     TAYMELNSLTSEDSAVYYCARXXXXXXDYWGQGATLTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPV
Published  TAYM????LTSEDSAVY-C?R????G?DYWGQGTTLTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPV

Valens     TVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIV . . .
Published  TLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSPRPSETVTCNVAHPASSTKVDKKIV
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Light Chain

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Valens     DVLMTQIPLSLPVSLGDQASISCRSSQXIVHXNGNTYLEWYLLKPGQSPKLLIYKVXNRFSGVPDRFSGSGSGTDFT
Published  DVLMTQ?PLSLPVSLGDQASISCRSSQ?IVH?NGNTYLEWYLQKPGQSP?LLIYKV?NRFSGVPDRFSGSGSGTDFT

Valens     LKISRVEAEDLGVYYCFQGXHXPYTFGGGTKLEIRRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWK
Published  LKISRVEAEDLGVYYCFQG?H?PYTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWK

Valens     IDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNR . . .
Published  IDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNR
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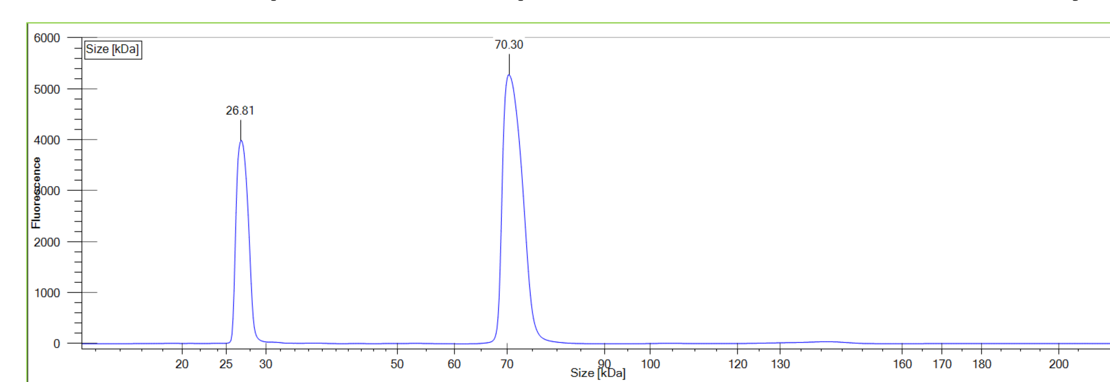
34 Unknown residues in the the published sequence (blue)
14 Amino acid differences (orange)
Some amino acids are replaced by 'X' for proprietary reasons.

Making Recombinant HM2 Antibody

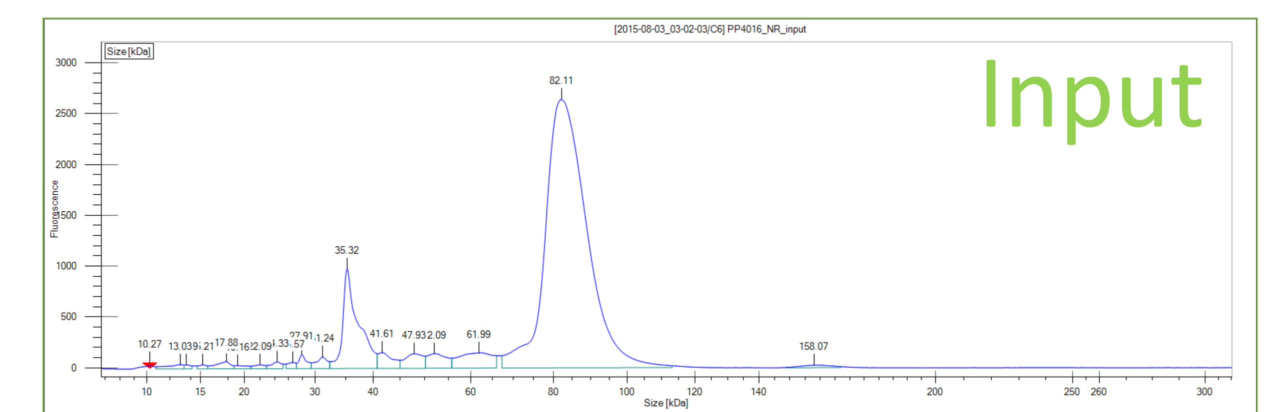
A recombinant antibody with human constant regions and the amino acid sequences identified by Valens as the variable regions were made. Plasmid DNA encoding the full length antibody was transfected to CHO cells to produce the HM2 antibody. HM2 antibody can be purified very efficiently from the conditioned media with Protein A beads. Purified HM2 antibody was tested for binding to multiple proteins in comparison to Sigma M2 antibody, and the results showed that they have similar binding profile and affinity (Kd).

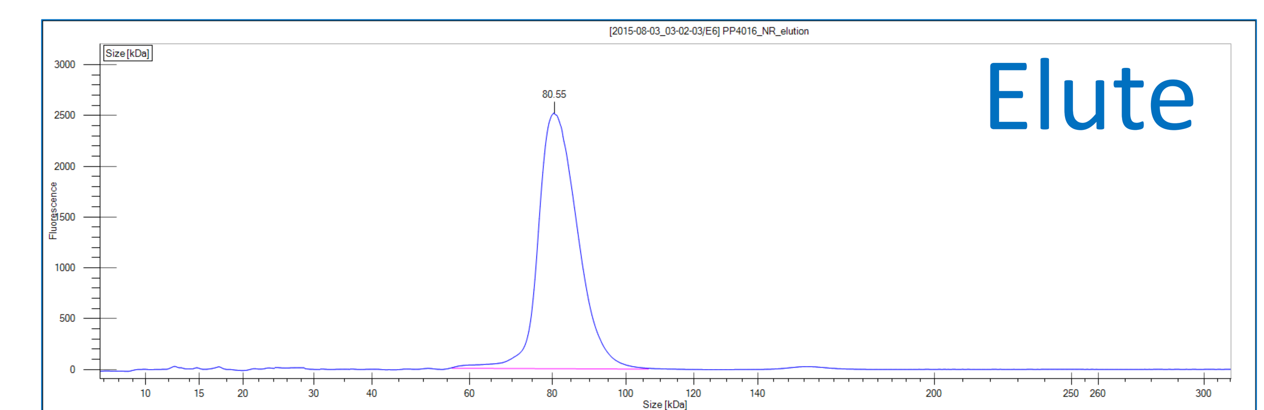
CE-SDS profile of purified HM2 antibody



	Kd against FLAG-tagged protein (Octet)	Kd against His-tagged protein (Octet)	Kd against 3x FLAG-tagged protein (Biacore)
HM2	26 ± 3 nM	No binding	< 1 nM
M2 (reference)	25 ± 4 nM	No binding	< 1 nM

Using HM2 For Purification





HM2 antibody was chemically conjugated to sepharose beads. The resulting beads were used to purify from a mixture containing a FLAG-tagged protein. As shown in the graphs above, the HM2 beads can specifically and efficiently purify FLAG-tagged proteins, with a recovery rate greater than 90%. The target protein binding capacity is 13.5 mg per mL of HM2 resin, which is at least 10x better than Sigma M2 resin.