Understanding the 3-D structures of a peptide to determine the control strategy for biological activity

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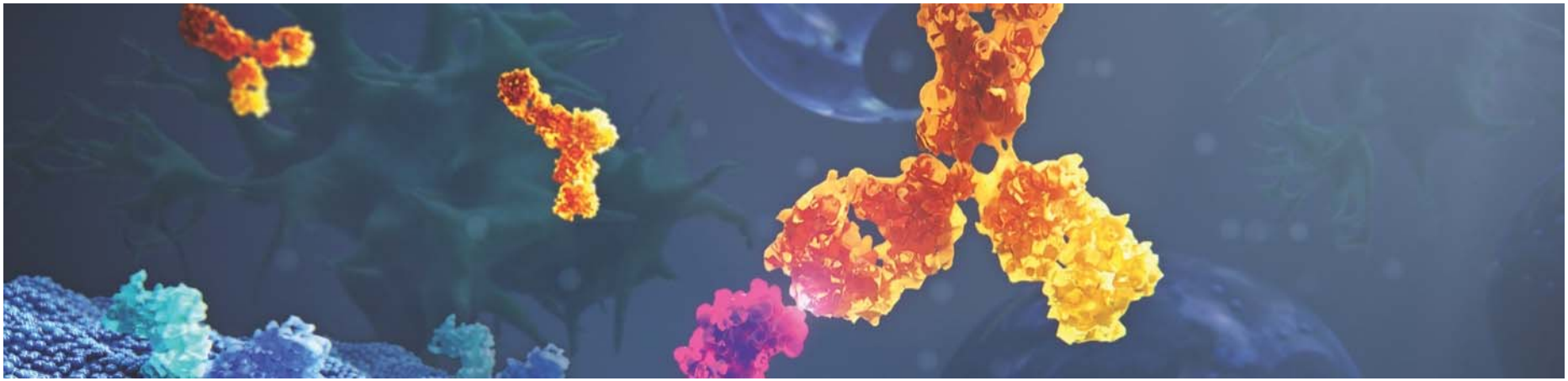


Introduction

- Context
- Analysis/Understanding
- Regulatory position and defence

Compound background

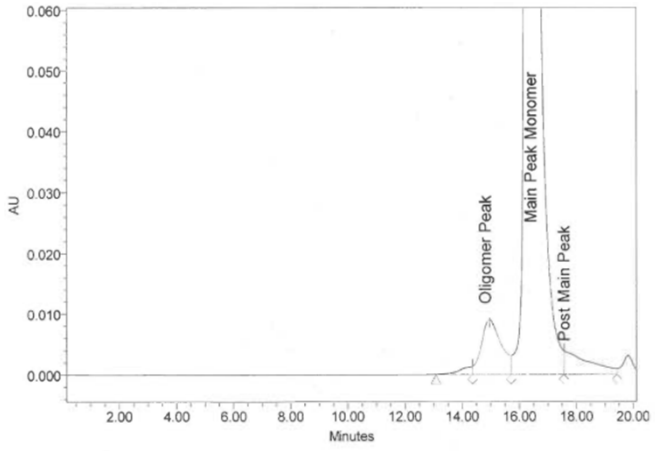
- AstraZeneca supported the acquisition by a third party in 2012
- Fully acquired by AstraZeneca in 2014
- Once weekly parenteral administration
- Drug product suspended in an oil
- Same API as existing immediate release and once weekly product with aqueous vehicles

Critical Quality Attributes (CQAs)

- Bioassay and Oligomers
  - Relate to **efficacy** and **safety** of the product
- Use understanding of the behaviour of the API (secondary, tertiary and quaternary structures) to develop control strategy for these attributes
- Experimental work performed by previous companies

Inherited position

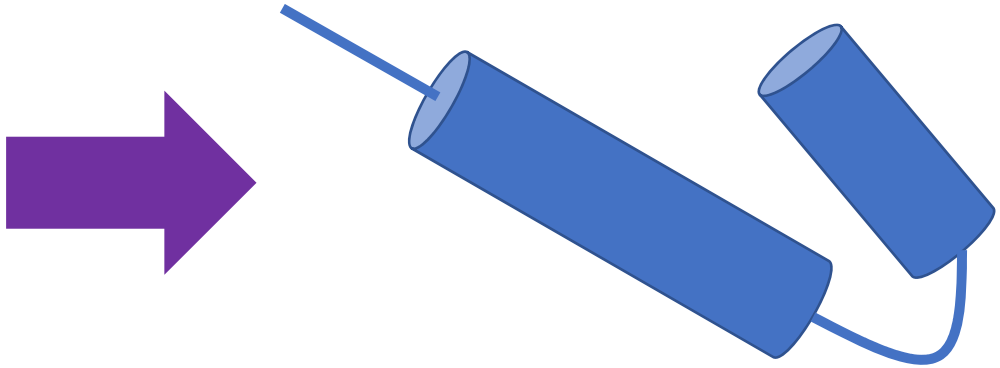
- Inherited data and some understanding of bioassay and oligomers for the product
- Methods available for Bioassay and 'Oligomers'
- Methods are complex and time consuming
  - No isolated sample of 'Oligomers' available so have to prepare each time



- Bioassay – on specification for some territories for immediate release product
- Oligomers; Size Exclusion Chromatography (SEC) method on specification for some territories for immediate release and once-weekly product
  - Evidence (Mass Spectrometry) that it can detect covalently bound dimers
  - Unclear whether method is capable of detecting non-covalently associated oligomers

Secondary and tertiary structures

- UV-Circular Dichroism (CD)
- NMR – structural modelling
- Temperature studies



Temperature studies demonstrated that forming and melting the 2°/3° structures were completely reversible

Quaternary structure

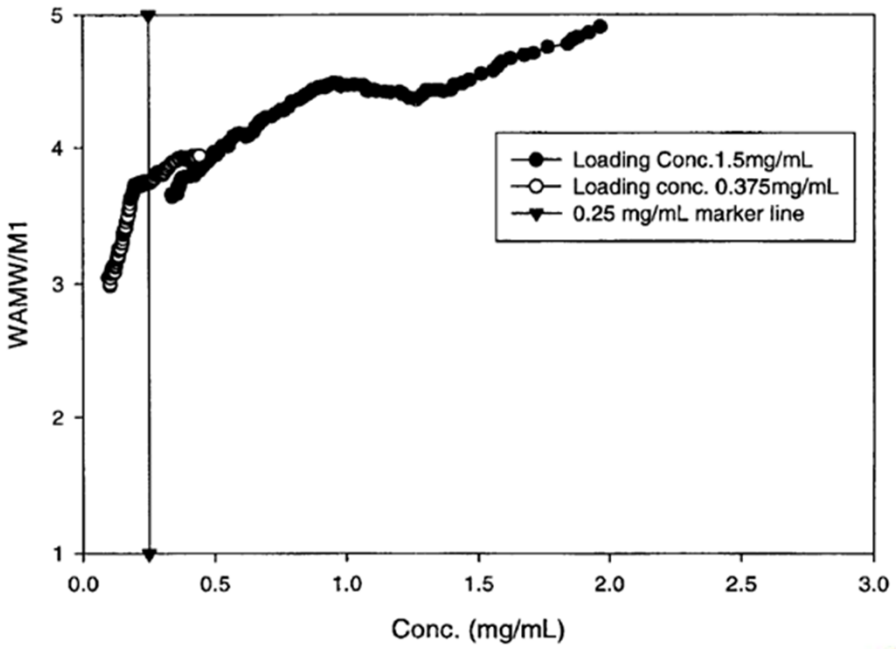
Techniques

Analytical Ultracentrifugation
Static Light Scattering
Dynamic Light Scattering
NMR Spectroscopy
Fluorescence Spectroscopy

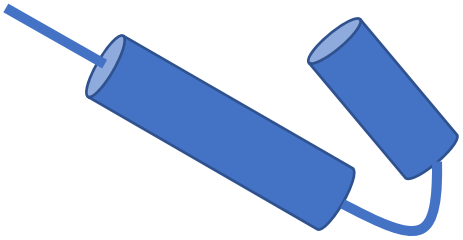
Variables

pH
Temperature
Concentration
Solvent

Analytical Ultracentrifugation



Quaternary structure



⇌ Non-covalent Oligomers } On average, across techniques, typically observed to be a tetramer

- Self association found to be reversible
- Note: possible to form a variety of covalently bound dimers - degradation

Bioassay position

- API is a relatively small peptide and as such would not be expected to have fixed 3-D structure (more like a small molecule)
- Circular Dichroism (CD) UV spectrum is the same before and after heating to 90°C, therefore, any conformational changes are completely reversible and shows no indication of cooperative unfolding (API does not become 'stuck' in an inactive conformation)
- No difference in Variable Temperature-CD profiles for API or API extracted from Drug Product
- Differences in bioassay before/after temp cycling are within the variability of the method
- No change in bioassay on stability

Oligomer position

- Release and Stability data suggest that this is not an issue in the (higher risk) immediate release product (method questions)
- Unlikely to form in extended release drug product during storage
- Reversibility of oligomer formation means equilibrium position will be achieved once released. Very low concentrations will also drive equilibrium position towards monomeric API.

Regulatory position

Enhanced understanding demonstrates that quality is assured without the need for a biological activity or oligomer testing clause for the new product

Why?

Bioassay: already removed from specification for existing once-weekly product, which was heavily cross referenced. Understanding above demonstrates it is low risk.

Oligomers: Inherited technical and regulatory positions somewhat contradictory due to different assessments/views of previous companies (e.g. China submission; value of SEC method – MS data; API isolation vs method solvents)

Considered a likely area of regulatory interest. API is a 'medium' sized molecule, but ultimately, it's behaviour is more like a small molecule => the risk to quality is low.

Regulatory questions

- 1 to date – justify absence of Bioassay clause
  - Responded and no further questions received

- Success!

Acknowledgements

Martin Kenworthy, Zahra Hamrang, Julie Ennis

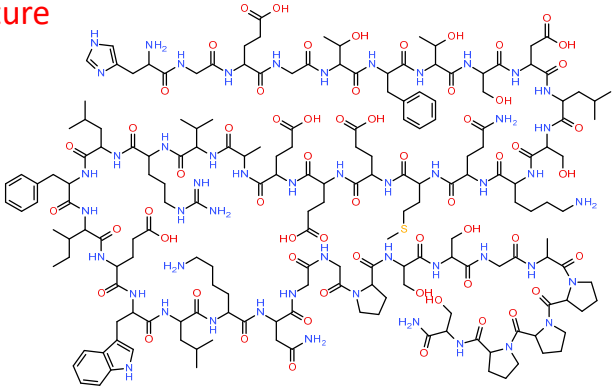
Thank you | Questions

Confidentiality Notice

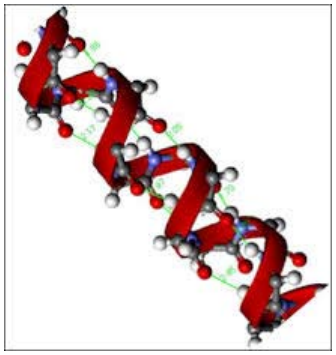
Backups

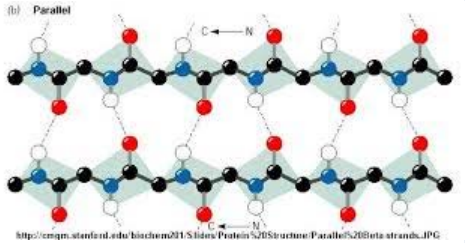
Higher order structure - definition

Primary Structure

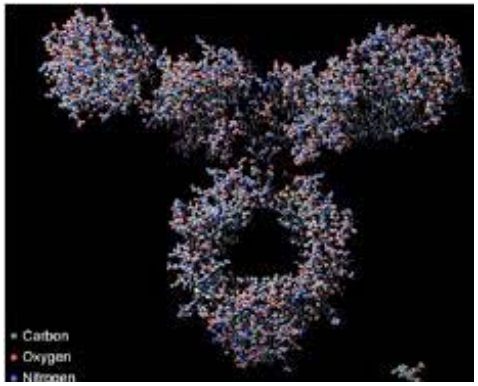


Secondary Structure





Tertiary Structure



Quaternary Structure