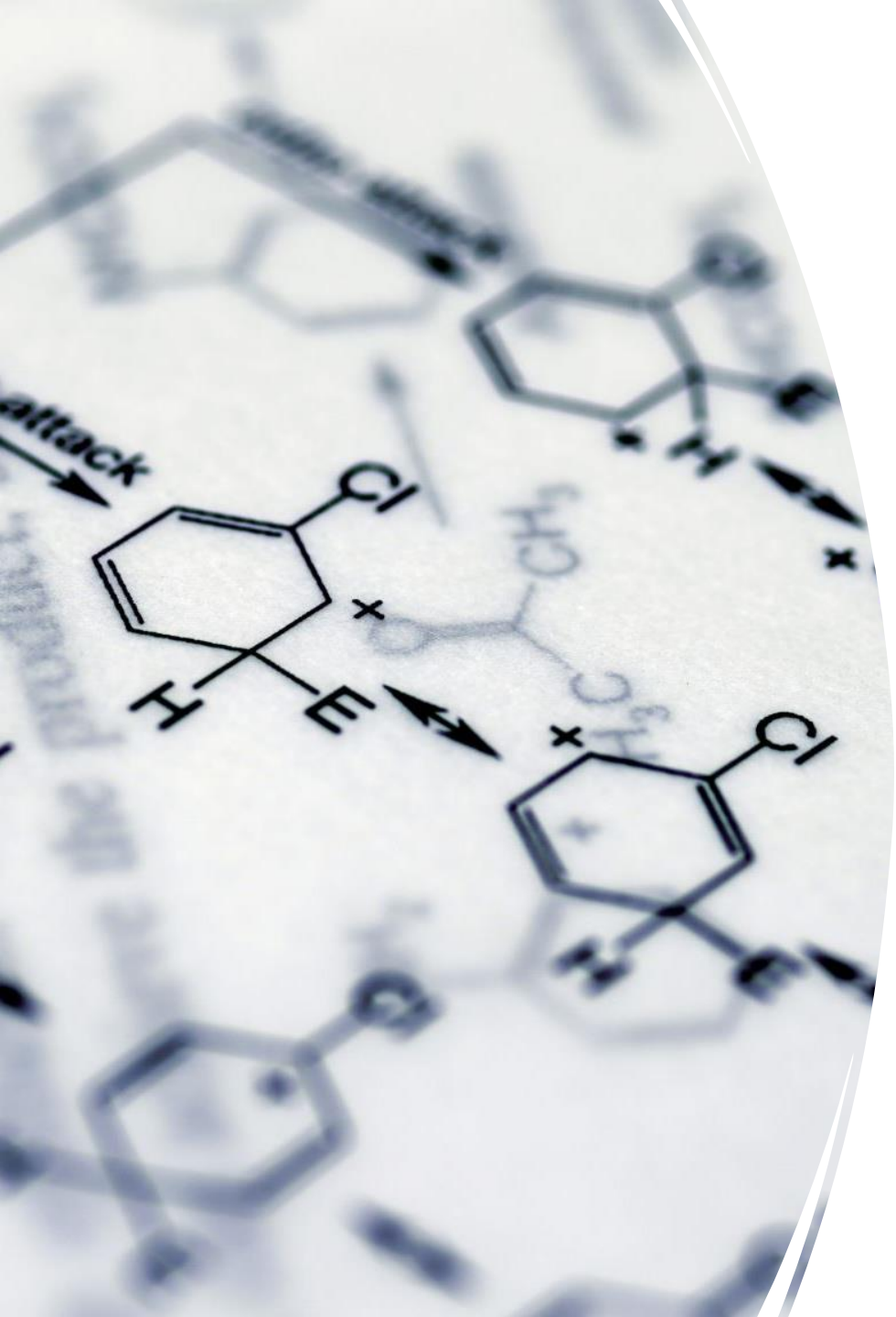

Case Study: Investigating Monoclonal Antibody Fragmentation Issues

Scenario

- Your company has successfully produced and marketed a monoclonal antibody for five years with no production problems or failures. Over this time, about 20 lots have been manufactured and have **met all quality standards**. However, the 21st lot, when placed on an annual stability monitoring protocol, shows a **higher-than-normal rate of fragmentation in the accelerated condition**. The subsequent next two lots also exhibit increased fragmentation.

- Your team has been assigned to investigate this issue and recommend the next steps.

Brainstorm Potential Root Causes

- Initiate QA investigation team (notify regulatory agency if appropriate)
- Review batch records
- Interview manufacturing and QC personnel
- Review QC records
- Identify potential product- or process-related root causes

Preliminary Lab Investigation Findings

- Mass spectrometry revealed that fragmentation was occurring at a specific point in the hinge region
- Protease inhibitors had no effect, but chelators restored expected stability
- All other physicochemical and biological tests were as expected
- Fe and Cr levels in all three affected lots were 2-fold higher than all previous lots
- Previous reference standard lot with a normal stability profile showed increased fragmentation when Fe was added but not Cr.

Stepwise Approach

Identify Potential Root Causes

- What are possible reasons for the increased fragmentation?
- Consider production process, formulation, storage conditions, and analytical methods.

Develop an Investigation Plan

- What steps should the company take to identify the cause?
- Who should be involved in the investigation (R&D, QC, Manufacturing, etc.)?
- What tests and data should be reviewed?

Propose a Corrective and Preventive Action Plan

- How can the issue be mitigated?
- What process or formulation changes might be necessary?
- How will you prevent recurrence?

Outcome

- Trace Iron Source
  - Compare metal ion profiles in all materials (raw materials, process intermediates, final product).
  - Test iron levels in equipment-contact materials (tanks, tubing, filters).
- Characterize Oxidative Stress Impact
  - Perform forced degradation studies to confirm oxidation as the primary mechanism.
  - Check for oxidation markers (e.g., methionine oxidation, tryptophan degradation).
- Process & Supplier Audits
- Review all process changes from lot 20 to 21 (supplier changes, material sources, equipment modifications).
- Perform on-site supplier audits if necessary.
- Mitigation Strategies
  - Implement tighter iron specifications for raw materials.
  - Consider adding chelators (e.g., EDTA) to formulation buffers.
  - Revise equipment maintenance and cleaning procedures.

References: Patent # US-20070110757-A1 “Antibody formulations having optimized aggregation and fragmentation profiles” and A.J. Cordoba et al. “Non-enzymatic hinge region fragmentation of antibodies in solution” J. Chromatogr. B 818 (2005) 115–121