

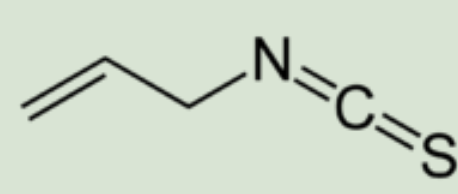
Covalent interaction of whey protein β -lactoglobulin with allyl isothiocyanate from Brassica vegetables

Possible consequences for the allergenic and inflammatory impact

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Introduction

- Bioactive compound from Brassica vegetables
- Antimicrobial
- Anticarcinogenic



AITC

Combination of **bioactive food constituents** and milk proteins with high nutritional value

Creation of **natural and safe nanocarriers** in food.

Covalent modification = high stability

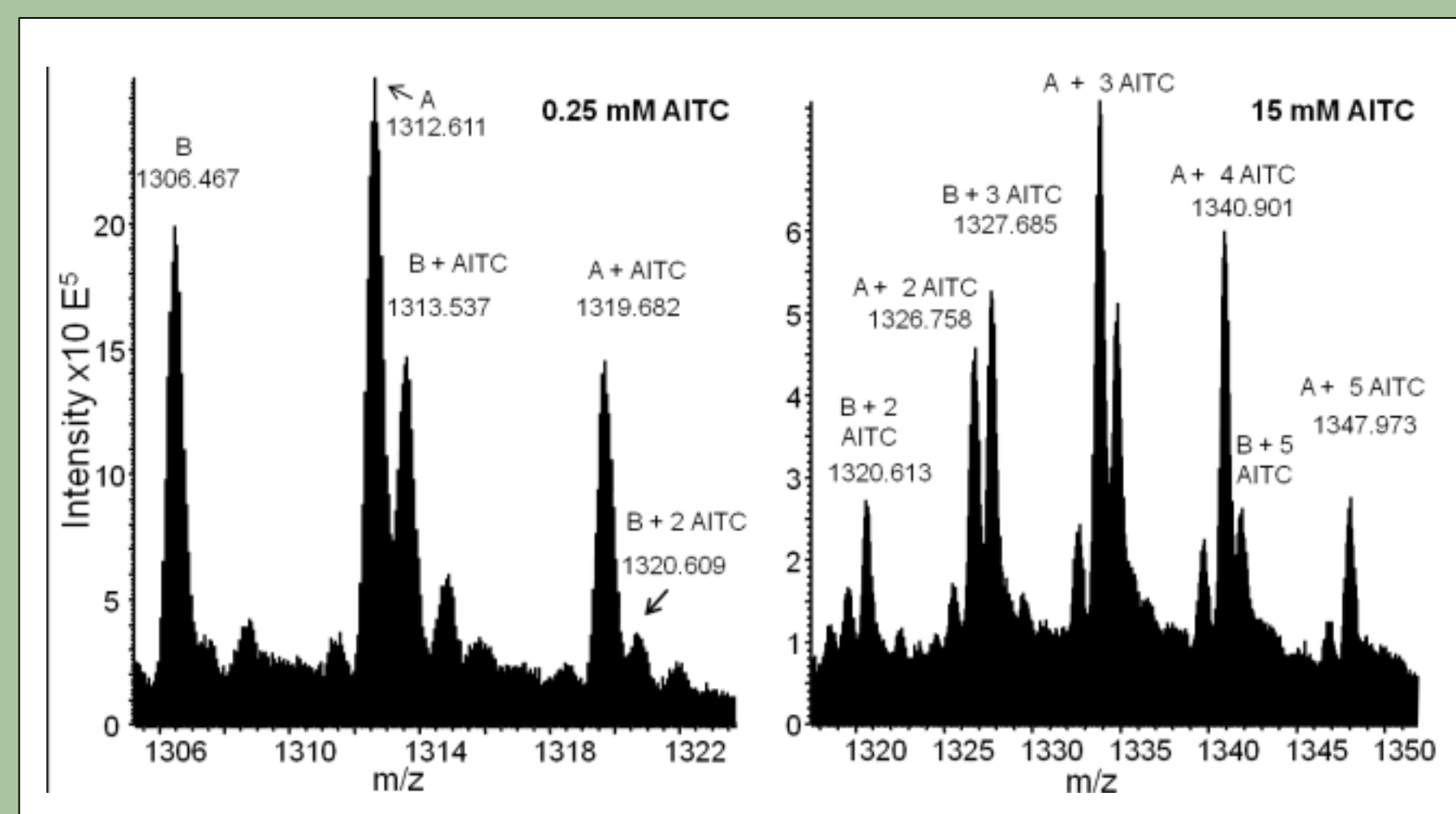
β -LG



- Whey protein
- pH-resistant pH 2-9
- Heat stable to 70 °C
- reaches the small intestine native + intact
- nano sized (4 nm diameter)

Experiments I

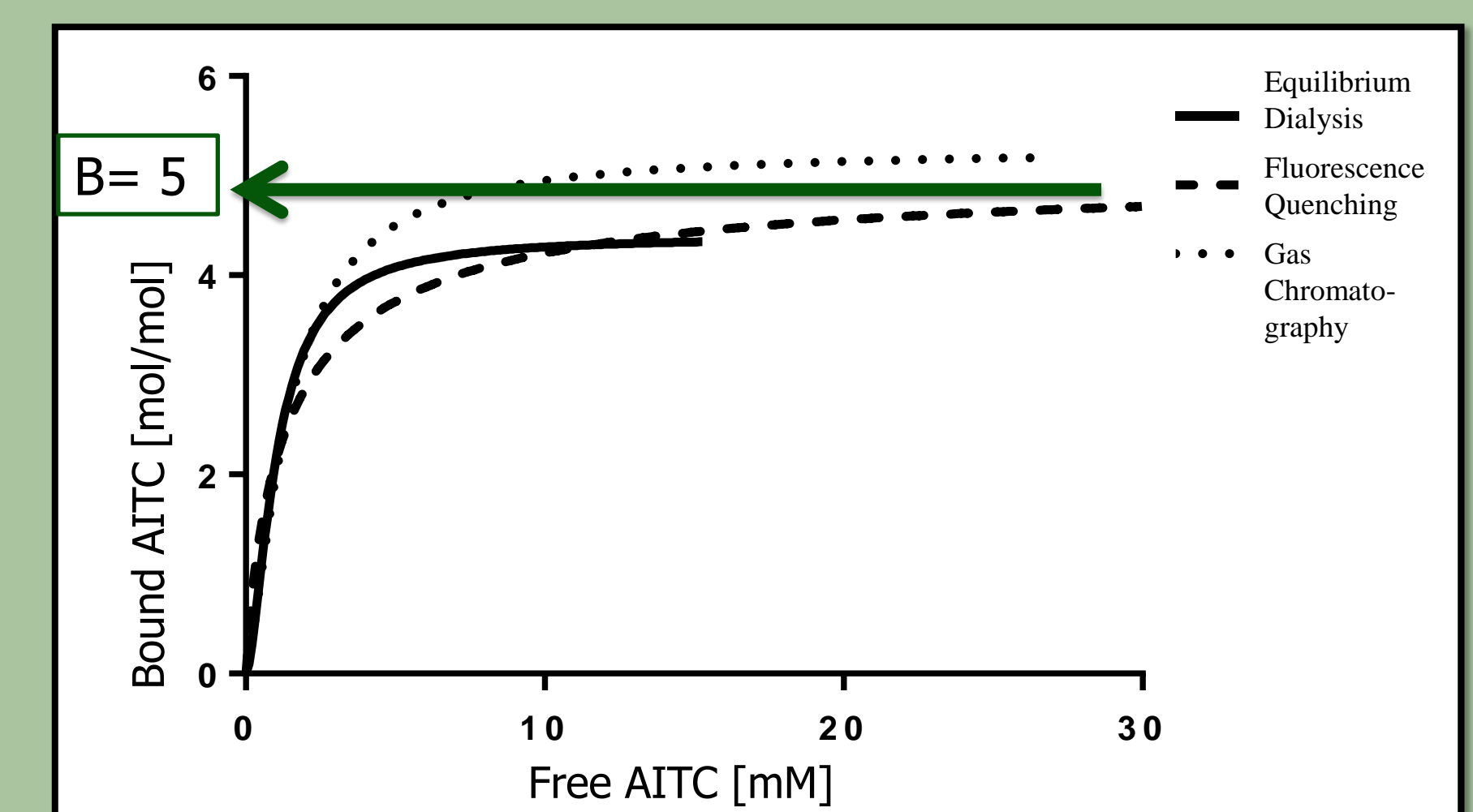
LC-ESI MS confirmed **5 binding sites (B)** on β -LG for AITC through mass differences.



Mass spectrometry results of 0.5 mM β -LG + 0.25 mM AITC, or + 15 mM AITC. A = β -LG genetic variant A, B = β -LG genetic variant B. At 0.25 mM AITC addition a maximum of 2 bound AITC were found, at 15 mM AITC addition 5 AITC were bound. m/z = mass/charge.

Experiments II

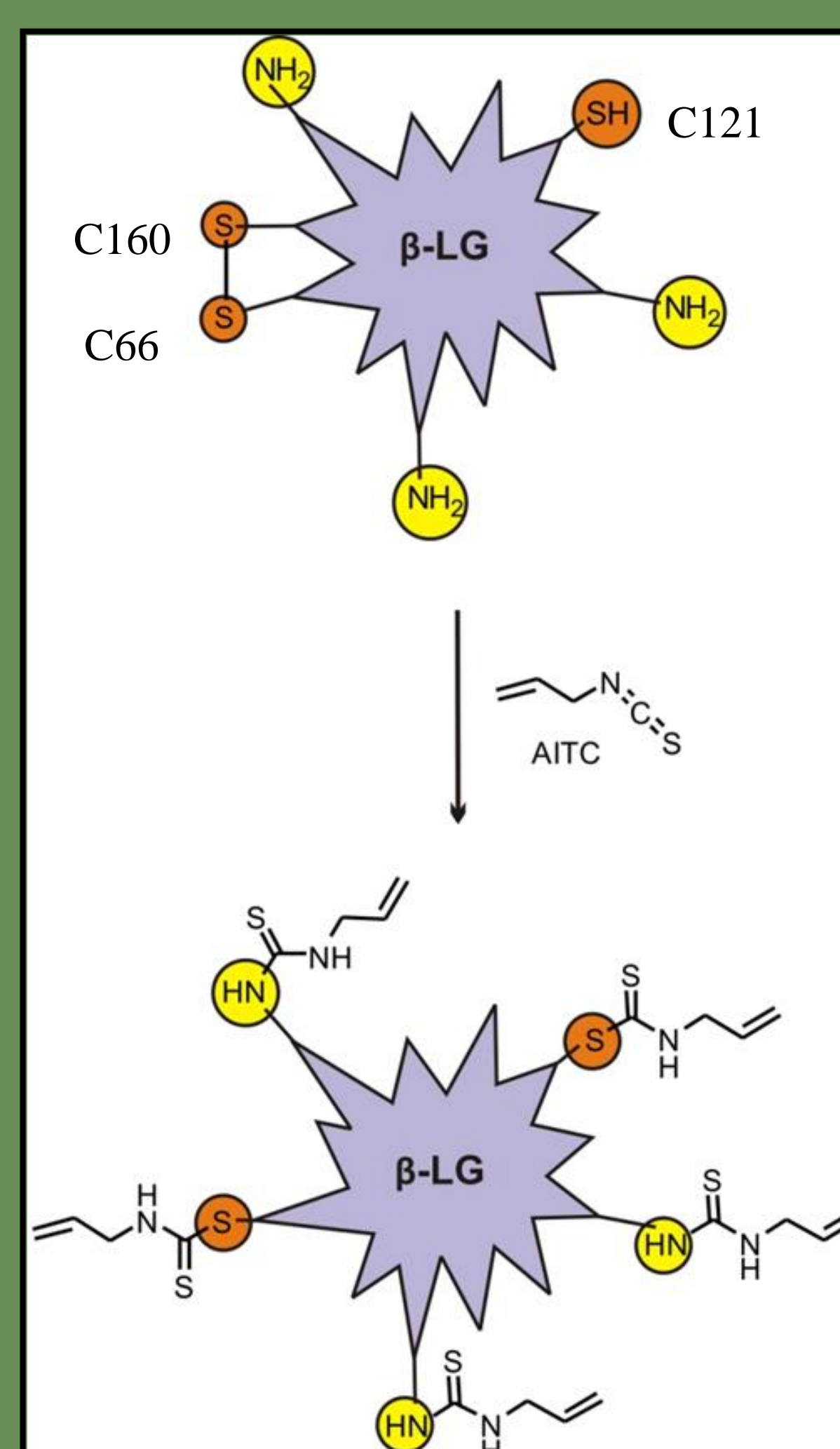
Physicochemical binding experiments all show **5 bound (B) AITC molecules** on 1 β -LG molecule:



Binding isotherm of 0.5 mM β -LG with increasing AITC concentration between 0 and 30 mM measured with ED, FQ or HWE. AITC = Allyl isothiocyanate, B = bound AITC [mol/mol].

Results

At pH 8.5



16 different AITC modification sites were found on β -LG, comprising of the following amino acid residues:

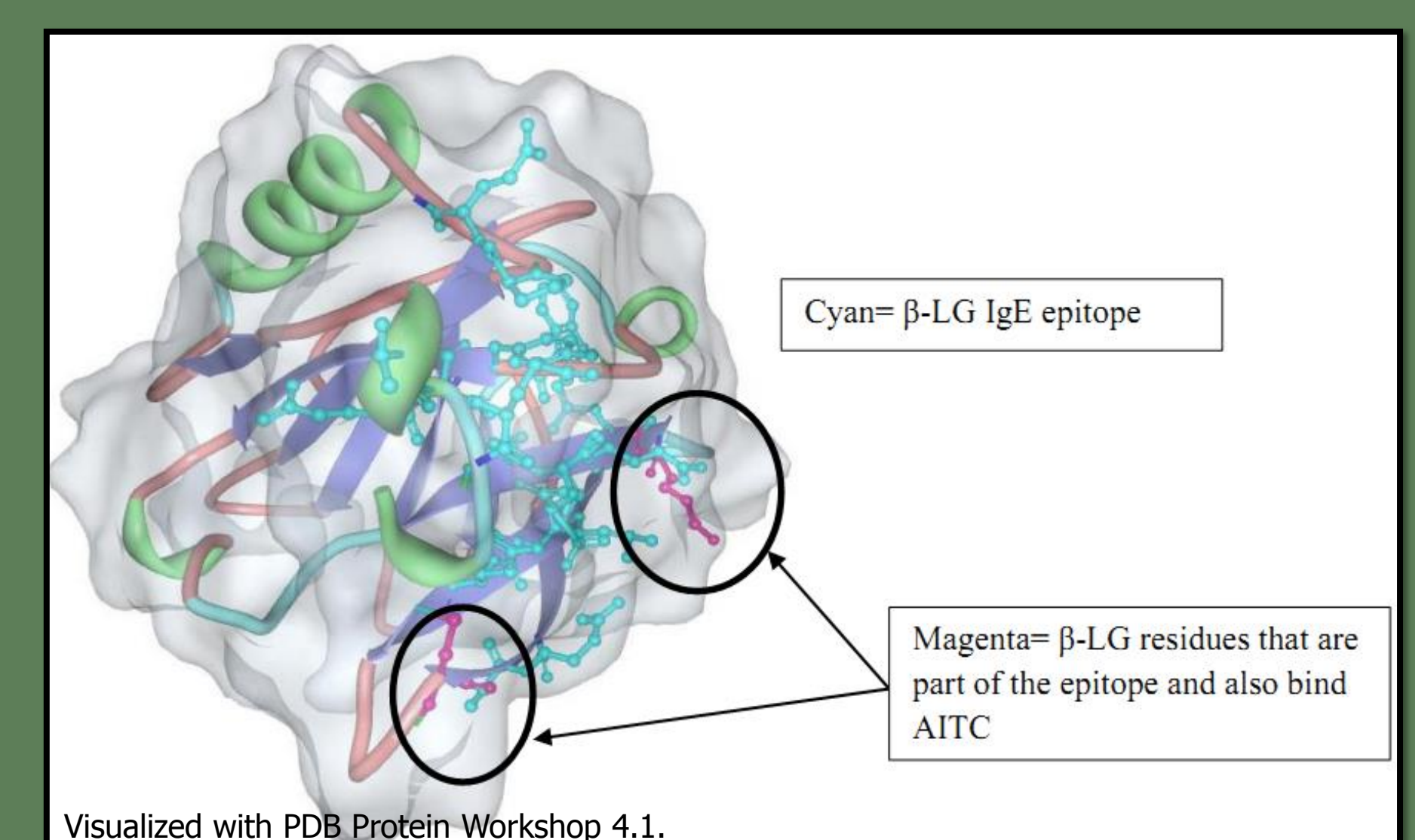
L1, K8, K14, K47, K75, K77, K83, K91, K100, K101, K135, K138, K141, C66, C121, C106

Of those **2 cysteines (C)** and **3 lysines (K) or leucine (L)** are modified simultaneously by AITC.

Resulting in a maximum of **5 binding sites**.

Influence on allergenic response?

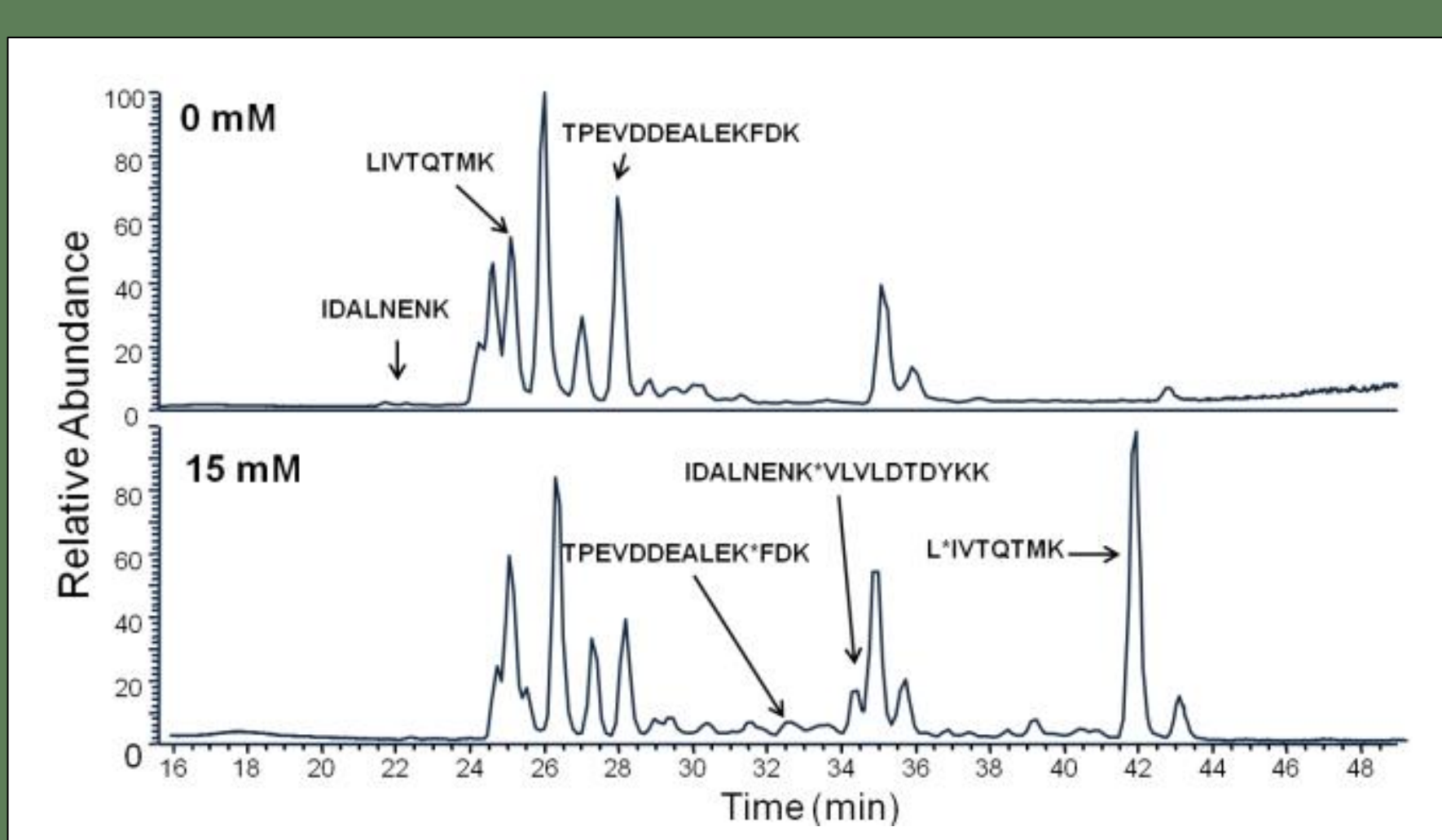
- β -LG is one of the **main allergenic agents** of cow milk
- Immunoglobulin E** epitopes on β -LG are well known
- AITC reacts** covalently with 2 amino acids that are part of the **epitopes**
- Covalent glycation at these amino acid residues **masks epitope recognition** for IgE
- Similar effects** are conceivable for AITC



β -LG amino acid residues that are part of Immunoglobulin E epitopes are marked in cyan, amino acids that are part of the IGE epitope and also interact with AITC are marked in magenta.

Influence on bioactivity?

- AITC addition changes the hydrolysis pattern of β -LG by **blocking cleavage sites**
- β -LG can also be hydrolysed to **bioactive peptides**
- Bioactive peptide concentration is not influenced** at lower AITC modifications.
- Both β -LG and AITC have **anti inflammatory activity**



Total ion chromatogram of β -LG (1 pmol) after tryptic digestion. β -LG without AITC (top) and with 15 mM AITC (bottom). Several peptide signals that decrease in signal intensity in "native" β -LG and increase in signal intensity in "labeled" β -LG are shown. AITC modified peptides elute at later retention times. * = labeled amino acids.

Conclusion - Covalent AITC binding :

- Changes digestion pattern of β -LG
- Changes protein conformation = disulfide cleavage
- pH dependent = regulation of complexation is possible
- Emulsifying capacity of β -LG at acidic pH increases

Prospect - Covalent AITC binding :

- Analyze IgE epitope blocking on allergenicity
- Test anti inflammatory response to protein bound AITC
- Analyze food technological and sensory changes of β -LG (increased foaming stabilization was reported and taste masking effects of AITC)



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