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Phosphorylation of breast-milk α_{S1}-casein induced conformational changes and abolished TLR4-agonisticity as well as formation of fibril structure

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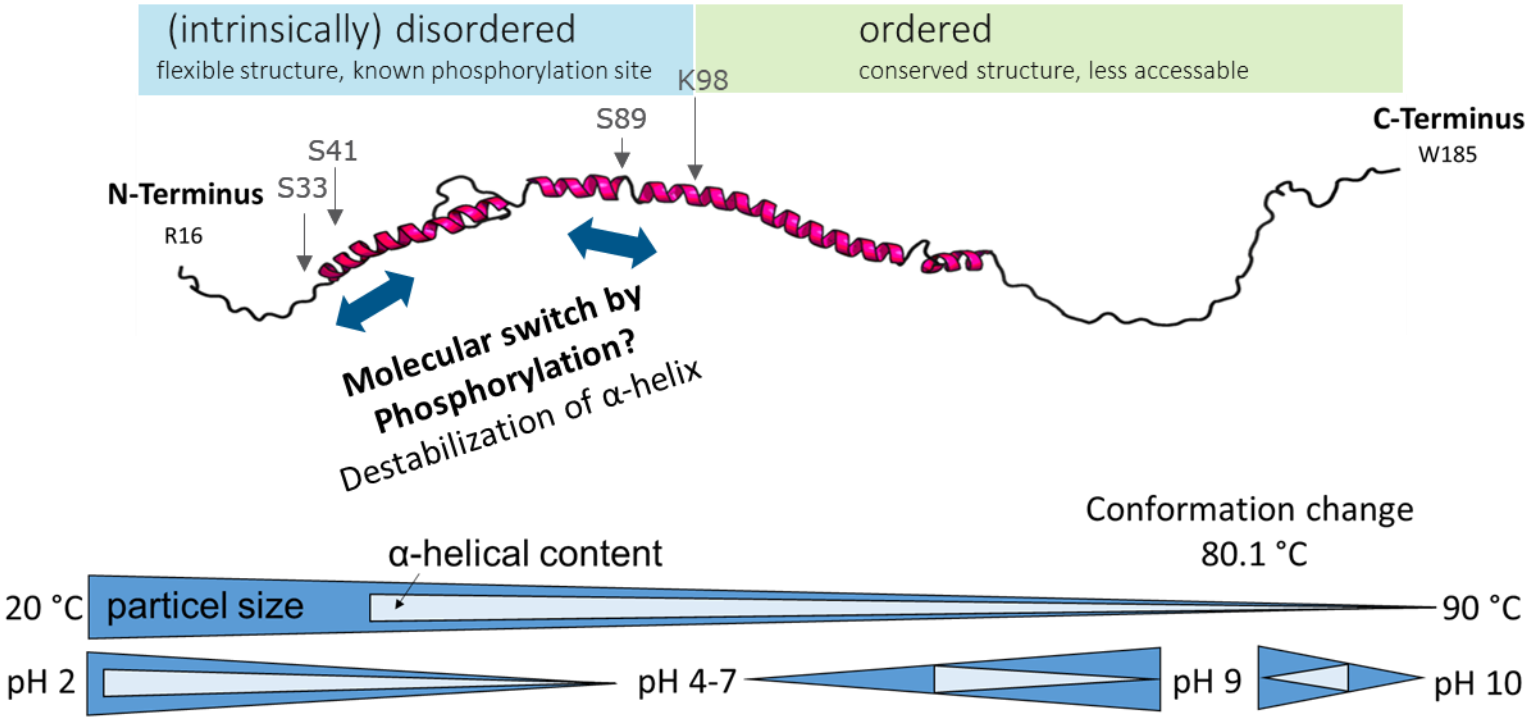
Graphical Abstract



Abstract: Breast-milk α_{S1}-casein is a Toll-like receptor (TLR4) agonist which induced proinflammatory cytokine secretion. Phosphorylated α_{S1}-casein (P-α_{S1}-casein) is non-agonistic. The objective of this study was to analyze structural characteristics underlying these observations.

Recombinant α_{S1}-casein was shown to exist in two conformations, an α-helical TLR4-agonistic conformation and a non-agonistic conformation with lower α helical and higher random coil content. TLR4-agonstic α_{S1}-casein conformation was found at a pH-range between 7.4 and 2. α_{S1}-Casein bound itself (KD-value: 2 µM) formed large aggregates (between Ø 73 nm [pH7] and Ø 826.2 nm [pH2]). Using Thioflavin T assay and atomic force microscopy showed that α_{S1}-casein adopted fibril-like structure. P-α_{S1}-casein was observed in a less α helical conformation, not inducing IL-8 secretion. P-α_{S1}-casein bound itself stronger (KD-value: 0.5 µM) than α_{S1}-casein and did not form fibrils.

In conclusion, TLR4-agonistic and non-agonistic conformations of α_{S1}-casein could be differentiated. It was demonstrated that human caseins are able to adopt fibril structure. These kind of structures are often disease related. We postulate, that phosphorylation could be a switch of two conformations regulating immunomodulatory effects of human α_{S1}-casein especially in immune system development.

Keywords: Breast milk; human α_{S1}-casein; TLR4 agonist; fibril structure, CK2.

Human α_{S1}-casein

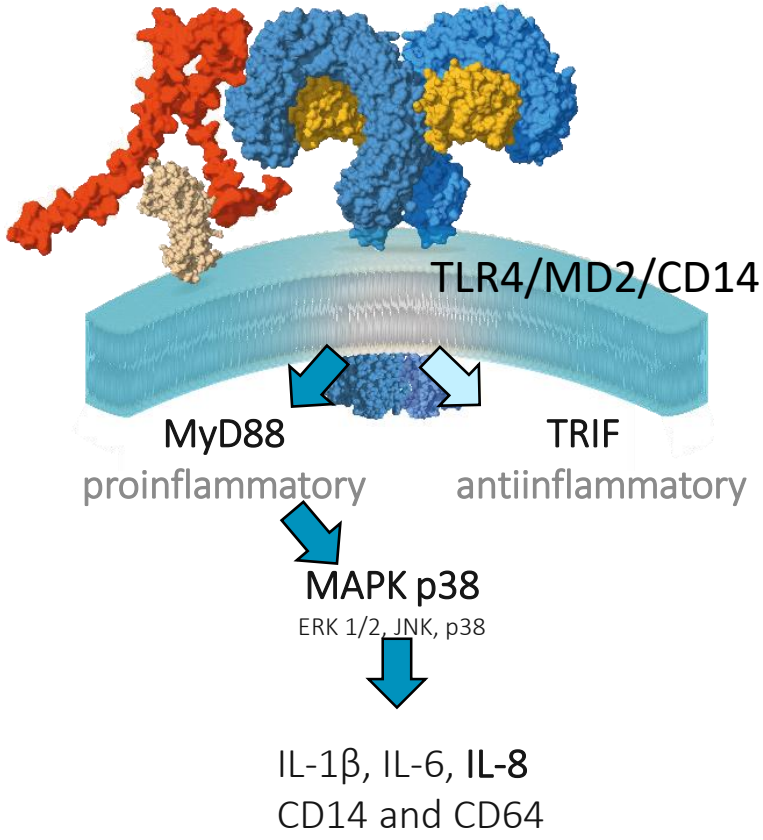
Expressed in:

- **Breast- and prostate cancer**
- **Synovia of patients** (arthritis)
- **breast milk** (functional food)
  transport of molecules, minerals
  induces life long IgG response

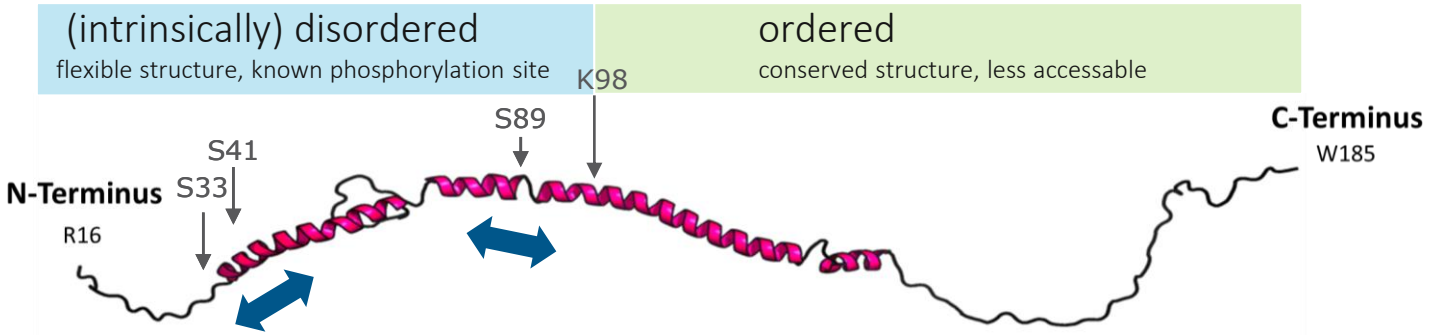
- **α_{S1}-casein bound** TLR4-receptors
- *In vitro* phosphorylated **α_{S1}-casein did not bind** TLR4-receptors

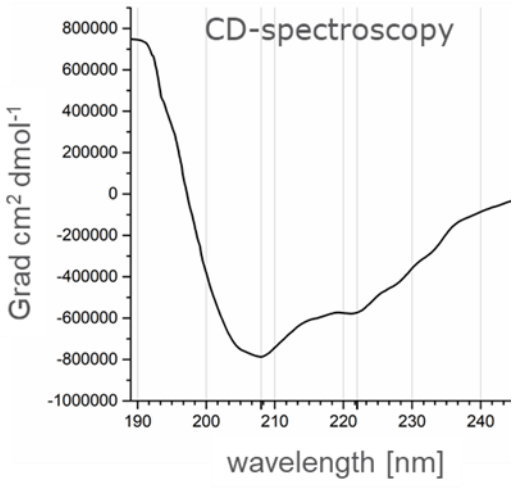
Is there a structure-function relationship for α_{S1}-casein activating TLR4?

Phosphorylation of α_{S1}-casein abolished this.



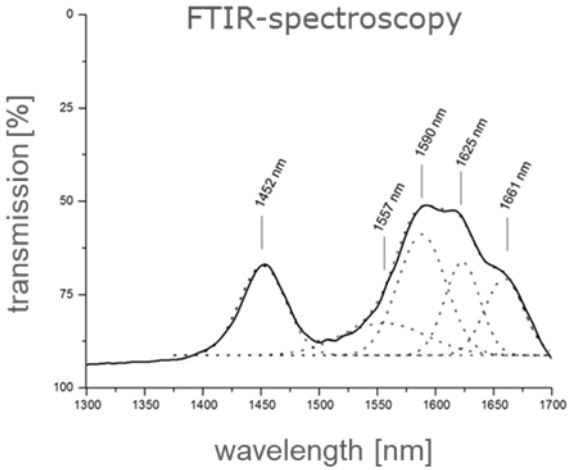
In silico predicted structure and *in vitro* analysis α_{S1}-casein



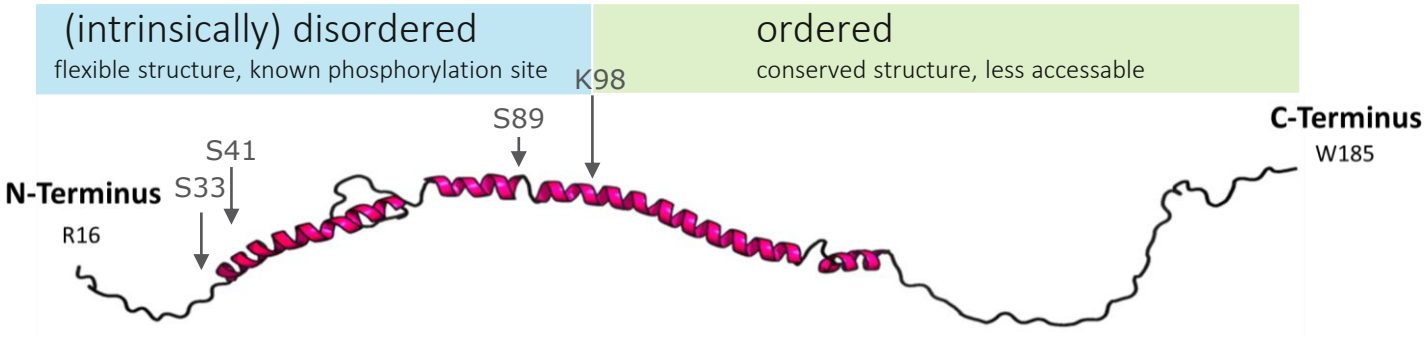


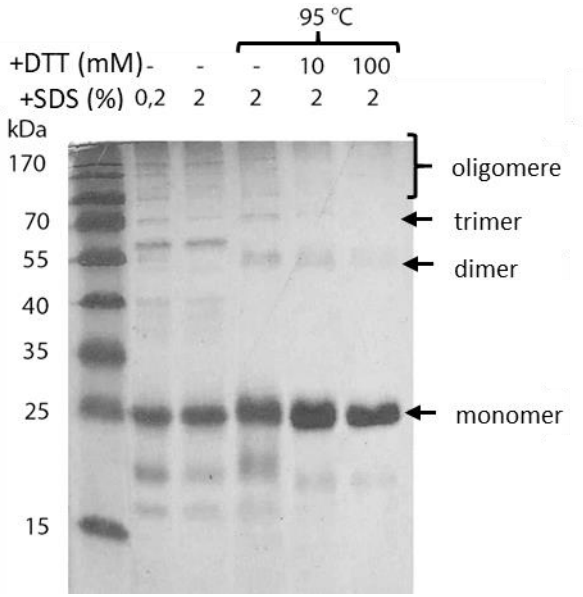
- **partial α-helical** structure
  1. ratio (222 nm/208 nm) of 0.74
  2. maxima at 1661 nm
- high intensity for used concentration (CD)
  → **result of multimerization?**

- maxima at 1625 nm (**β-sheet?**)

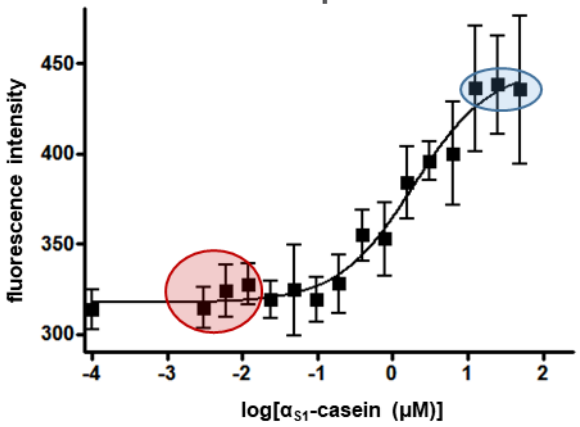


α_{S1}-casein binds itself?





Microsale Thermophoresis



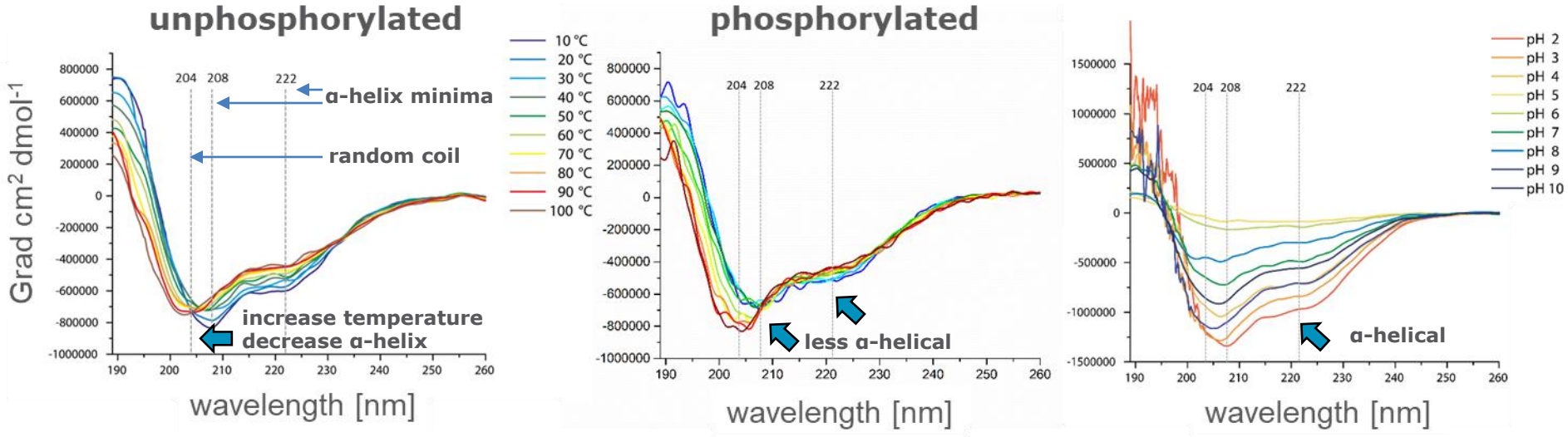
- Homomers
- „K_D-value: 2.2 µM“
- Diameter of particels: 73.4 nm (PI: 0.6)

➢ **α_{S1}-casein binds to itsself**

Correlation of α-helical structure and effects *via* TLR4

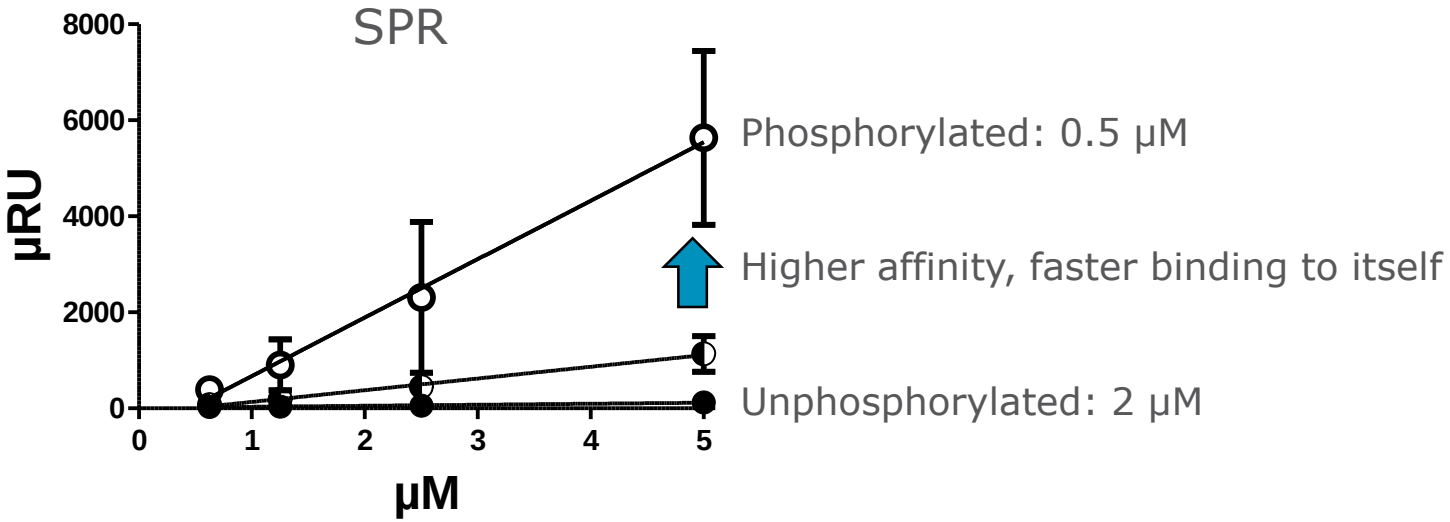
IL-8 sercretion via TLR4

- RT, pH7: **yes**
- 95 °C: **no**
- Phosphorylation: **no**
- pH2: **yes**



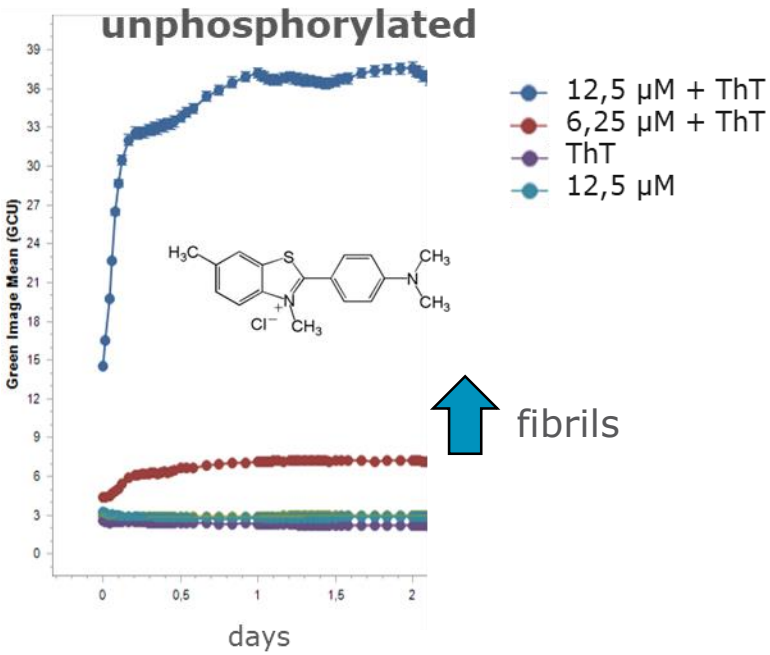
- α_{S1}-casein had higher α-helical content at RT (pH7 and pH2) than phosphorylated and heated one

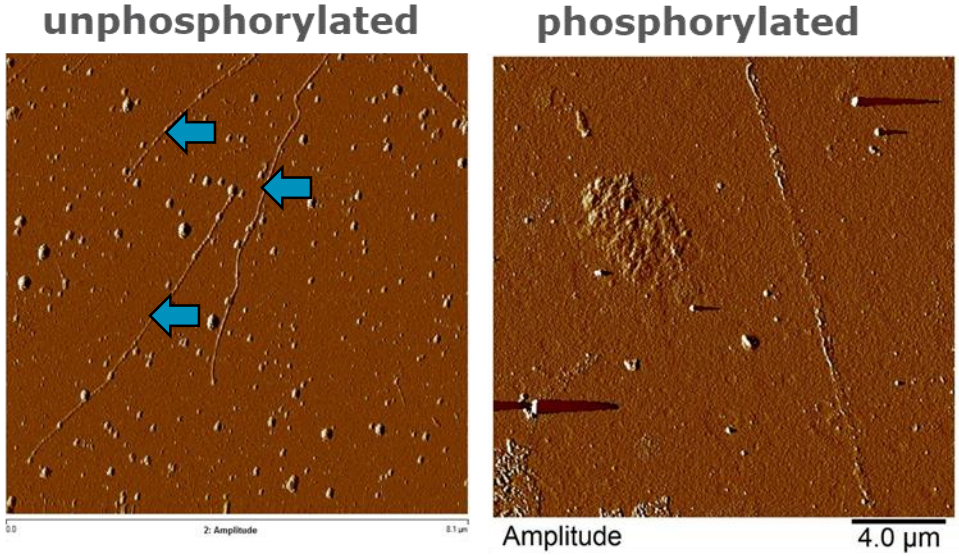
Difference in binding of α_{S1}-casein to itself



- Phosphorylation could be a mechanism to control multimerization
- Unphosphorylated: slower, structured
- Phosphorylated: faster, unstructured

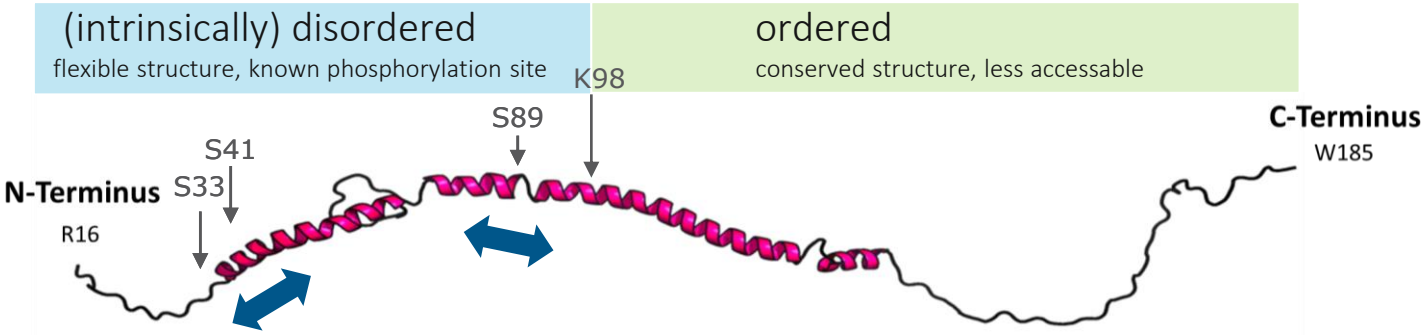
β-Sheet content and multimerization hint that α_{S1}-casein could form fibril structures





- Unphosphorylated α_{S1}-casein formed fibrils (shown by Thioflavin T Assay and AFM)
- Phosphorylated α_{S1}-casein did not form fibrils, but aggregates.

Conclusions



- α_{S1}-casein was shown to have two conformations, an α-helical TLR4-agonistic and a non-agonistic conformation with lower α helical content.
- Phosphorylation of α_{S1}-casein as well as incubation at 80 °C led to the non-agonistic conformation.
- β-Sheets and aggregation allowed us to identify fibril-like structures of specifically for α_{S1}-casein by ThT-assay and AFM
- phosphorylation could be a switch between two conformations of α_{S1}-casein regulating immunomodulatory processes of the immune system

Acknowledgments

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