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Challenges associated with *in situ* small angle scattering methods for semi-solid casein gel investigations

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INTRODUCTION

- The weak interaction between neutrons and matter allows neutrons to penetrate materials easily and as a result, it is feasible to use complex sample environments for *in situ* studies¹.
- Several flowing *in situ* digestion measurements used **scattering techniques to study liquid-like systems**. Although due to the experimental restrictions of neutron scattering, such as fitting solid samples in a sample cell, the time-dependent **behaviour of solid-like food structures are experimentally more challenging** due to the inability to study them in real time.

Casein protein provide the underlying structure of most dairy-based products, such as cheese and yoghurt. To understand casein gel behaviour during digestion, a real time measurement is needed across ultra-small (USANS) and small angle neutron scattering (SANS) (Fig. 1).

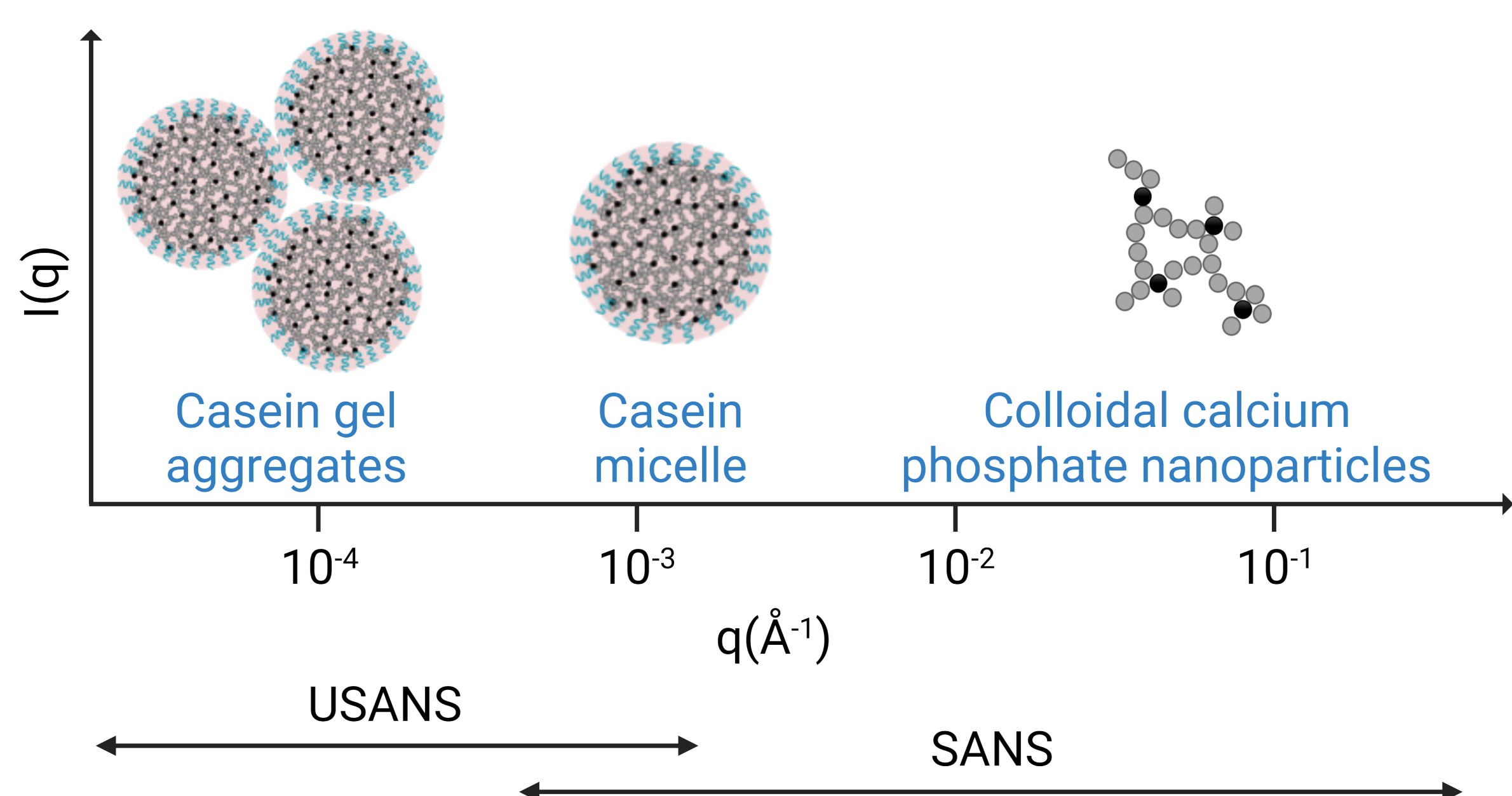


Figure 1 Schematic representation of USANS and SANS q range covering a casein gel from micro to nanoscale

To investigate a large casein gel particle during digestion, the gel should be under the neutron beam within a 1 mm cell maximum in order to avoid a phenomenon called 'multiple scattering'. New *in situ* setups for real time structural characterization during digestion is needed for neutron scattering.

RESULTS & DISCUSSION

In our recent study (Bayrak et al., 2021), the casein gels were physically disrupted into particles small enough to fit inside a 1 mm sample cell for the *in situ* flow system.

Blending gels to use the flow setup is found to be the most effective method for ensuring that the internal structure of gel particles does not change between the 1 nm and 20 μm length scales².

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- Imae, T., Kanaya, T., Furusaka, M., & Torikai, N. (Eds.). Neutrons in soft matter. John Wiley & Sons. (2011).
- Bayrak, M., Mata, J. P., Whitten, A. E., Conn, C. E., Flourey, J., & Logan, A. Physical disruption of gel particles on the macroscale does not affect the study of protein gel structure on the micro or nanoscale. Colloid and Interface Science Communications, 46 (2022) 100574.

EXPERIMENTAL DESIGN

For an *in situ* analysis of a gel structure during digestion, three options were considered: (A) **attaching the gel structure** to the surface of the sample cell or holding it within a mesh while passing digestive enzymes through, (B) trapping the gel in a **Teflon grid**, or (C) **blending** the gel particles (Fig. 2).

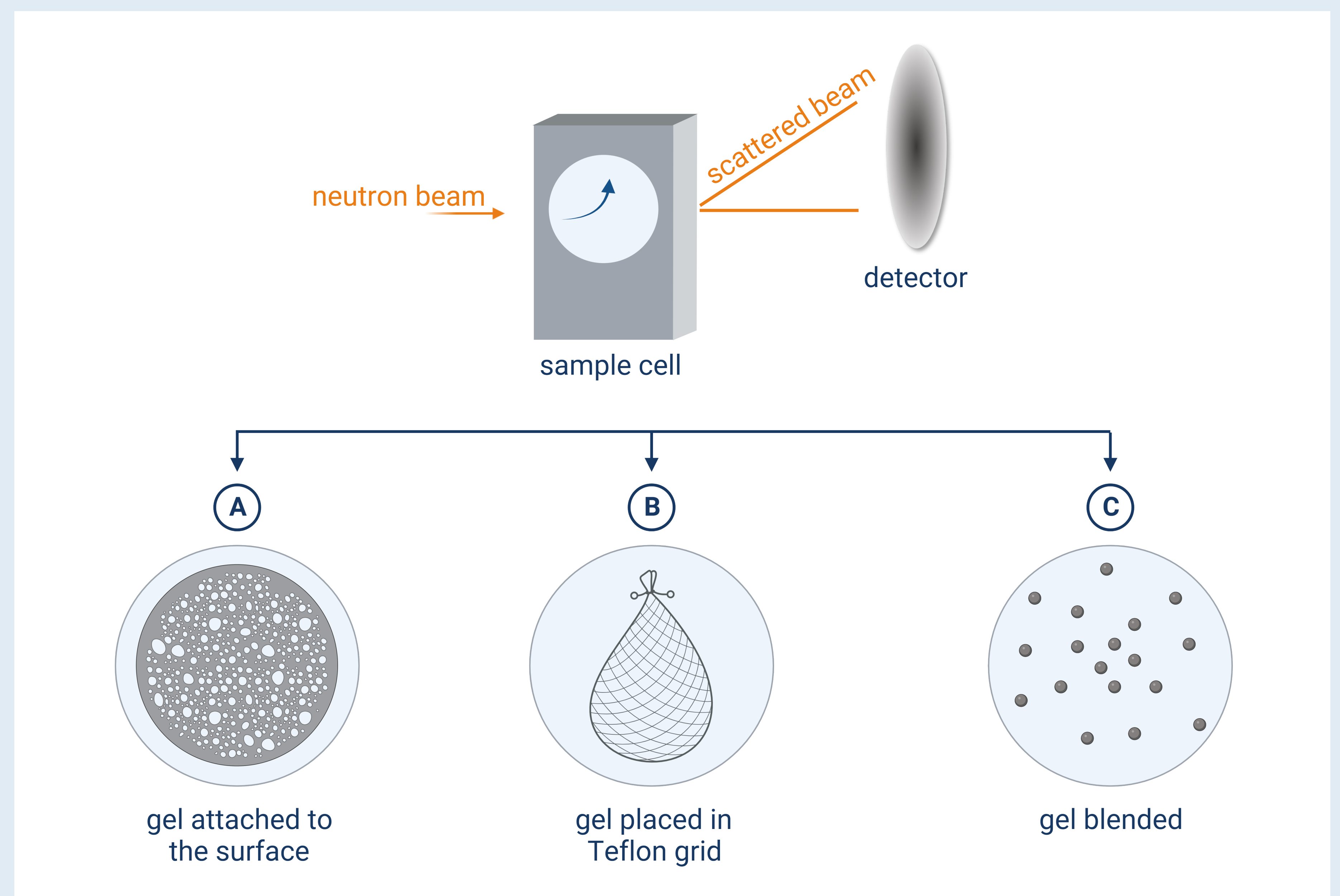


Figure 2 Options for evaluating the semi-solid casein gel structure *in situ* using USANS and SANS while digestive fluid is flowing

(A) There is an experimental thickness limit for casein gels to adhere to the silica surface of the sample cell; if they are produced too thin, they will not adhere to the sample cell surface. Given that the sample cell's maximum thickness is 1 mm, the gel must be thinner than 1 mm to allow digestive fluid to pass through. In addition, the movement of digestive juices may cause the gel to detach from the surface.

(B) Teflon, a material that does not scatter neutrons and is therefore invisible, might be employed to trap the casein gel. A Teflon teabag could hold 1.5 - 2g of a casein gel inside while digestive juices circulate around. In this instance, however, casein gels may be surrounded by insufficient volume of digestive fluid to observe rapid digestion and detect any structural change within the given timeframe.

(C) Physical disruption of larger gels into smaller gel particles, such as blending, appeared to be the simplest method. However, it should also be demonstrated whether there would be an impact on the micro- and nanostructure of gels.

CONCLUSION

Following blending of gel particles, the absence of a change in the structural properties of USANS and SANS indicates that the gel network structure was not affected at nano (CCP), micro (micelle) and macro (micellar gel) scales.

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