

RECOMBINANT CALPROTECTIN AS A PROMISING TOOL TO HARMONIZE MRP-8/MRP-14 IMMUNOASSAYS

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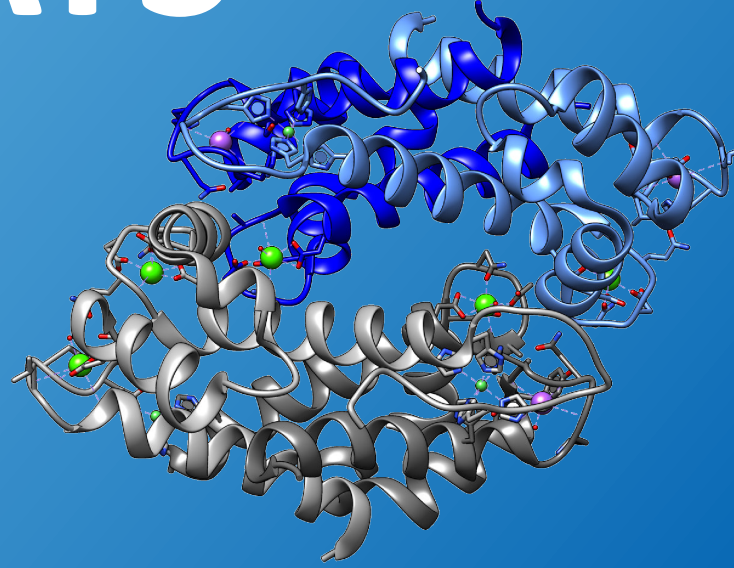
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Motivation

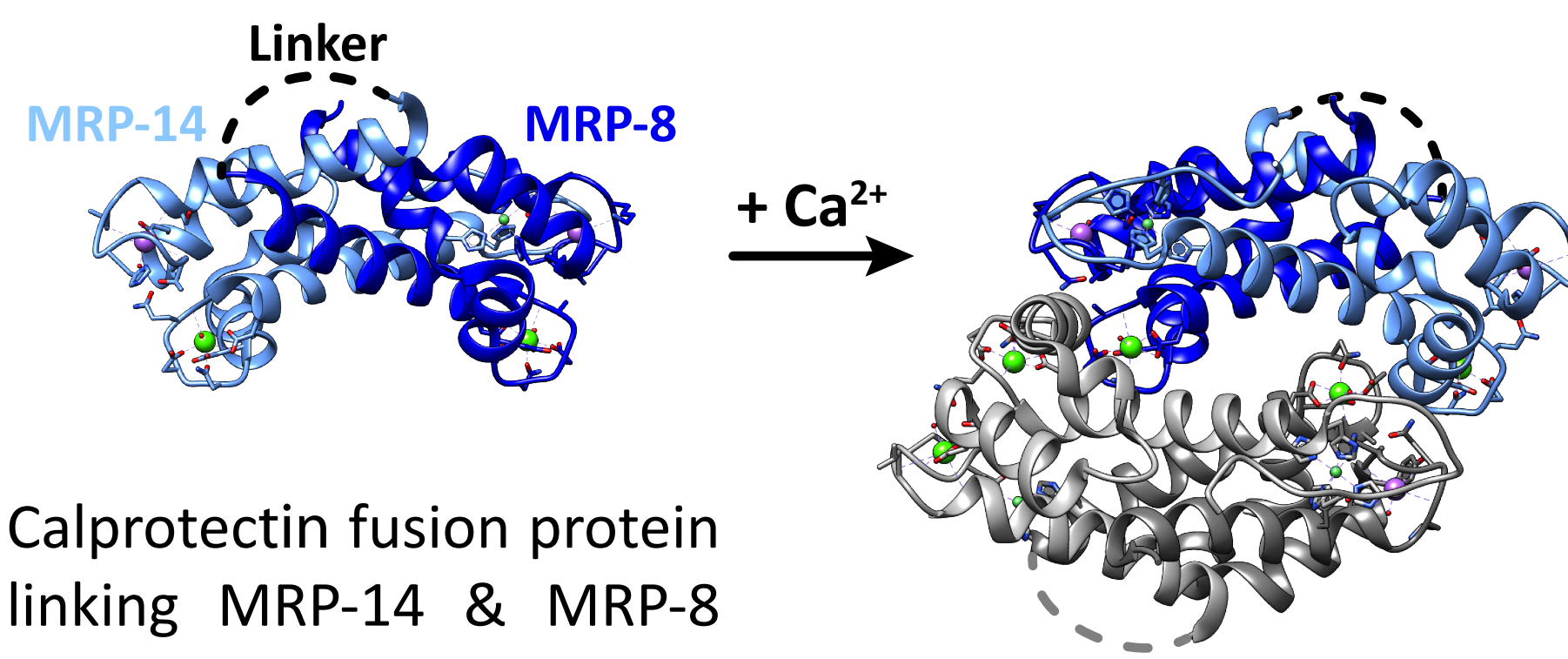
Calprotectin is a major granulocyte-derived alarmin protein natively occurs as a dimeric and tetrameric MRP-8/MRP-14 complex.

While serum calprotectin is an emerging biomarker for rheumatoid arthritis and juvenile idiopathic arthritis, fecal calprotectin is already the gold standard for diagnostics and monitoring of inflammatory bowel diseases. However, standardization of fecal calprotectin assays differs significantly among providers leading to varying clinical cut-offs.

One suspected reason is that calprotectin's oligomeric states can generate different quantitative results yet trapping calprotectin in a distinct oligomeric state is challenging. It is therefore required to produce pure calprotectin as calibrator material with a controllable oligomeric state

Recombinant calprotectin

RCAL



Calprotectin fusion protein linking MRP-14 & MRP-8 with a 3C-cleavable linker.

Calprotectin dimer

Calprotectin tetramer

Expression & Purification

Soluble bacterial expression

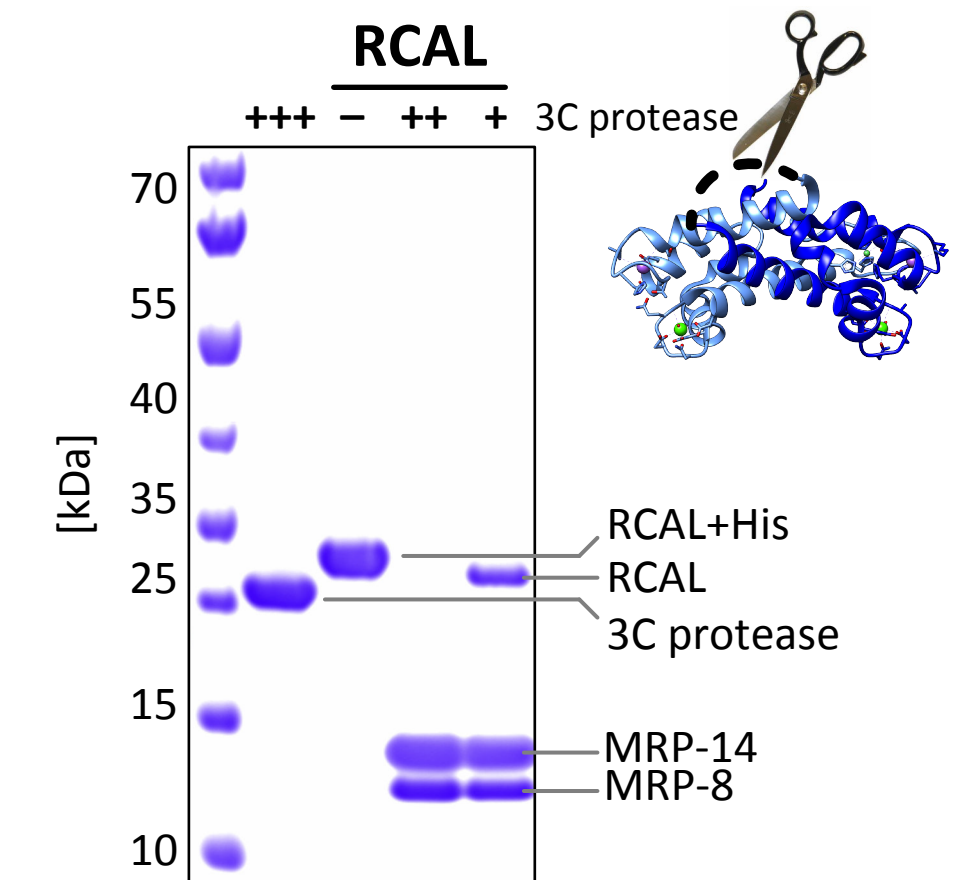
Ni-NTA column

optional: Anion exchange column

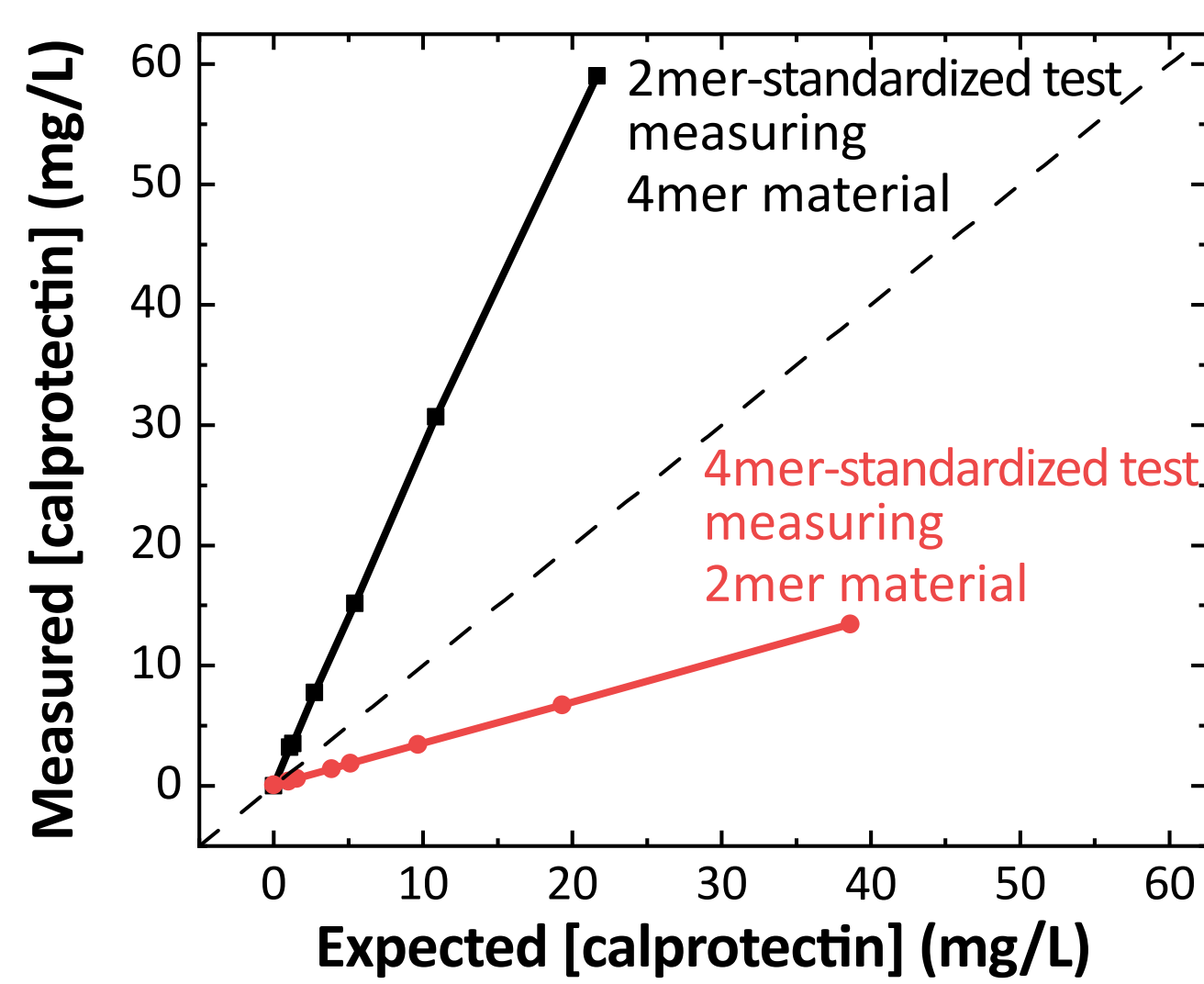
Size exclusion chromatography

optional: 3C protease cleavage

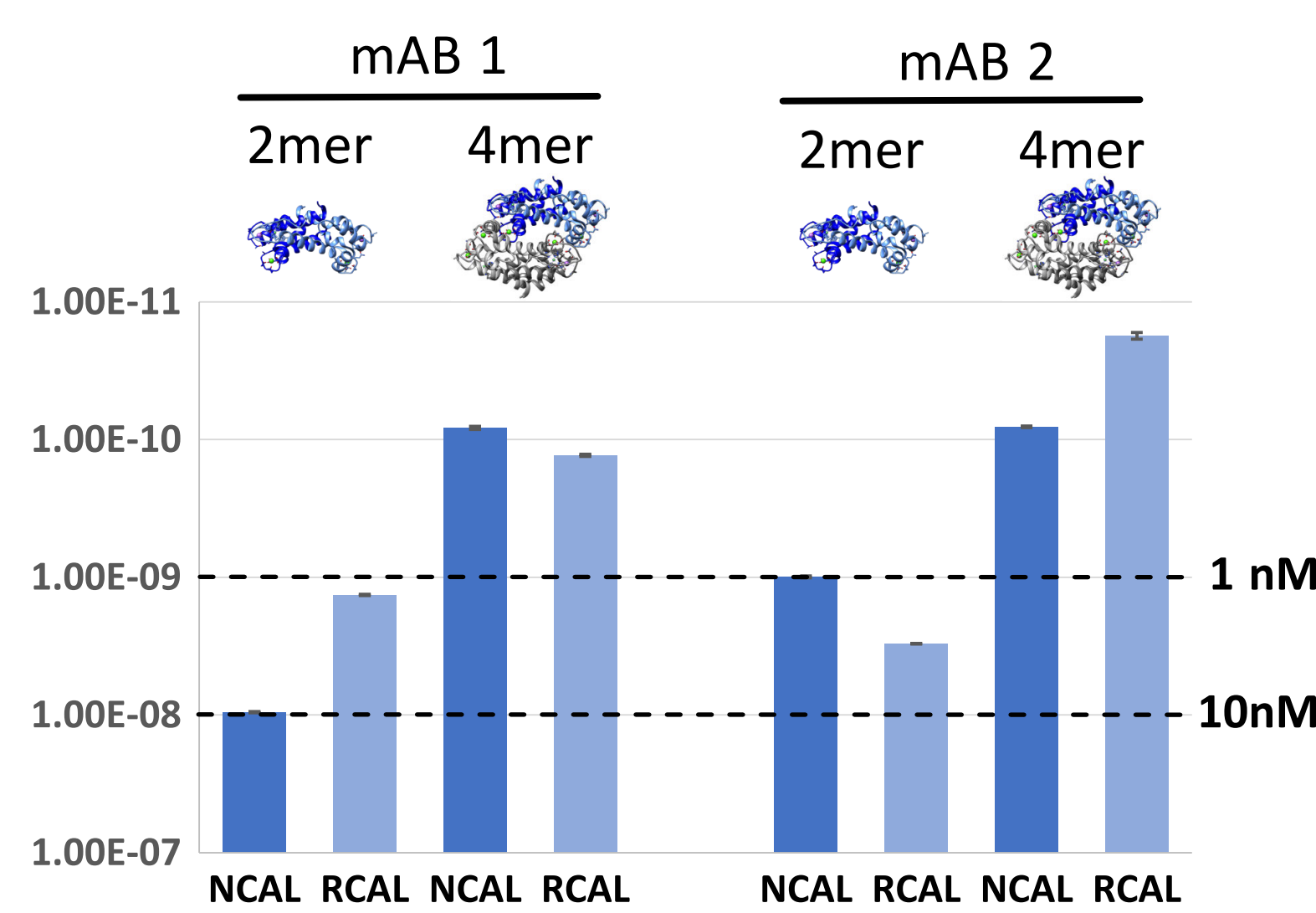
optional: Ca²⁺-induced oligomerization



Standardization & binding affinities depend on calprotectin's oligomeric state

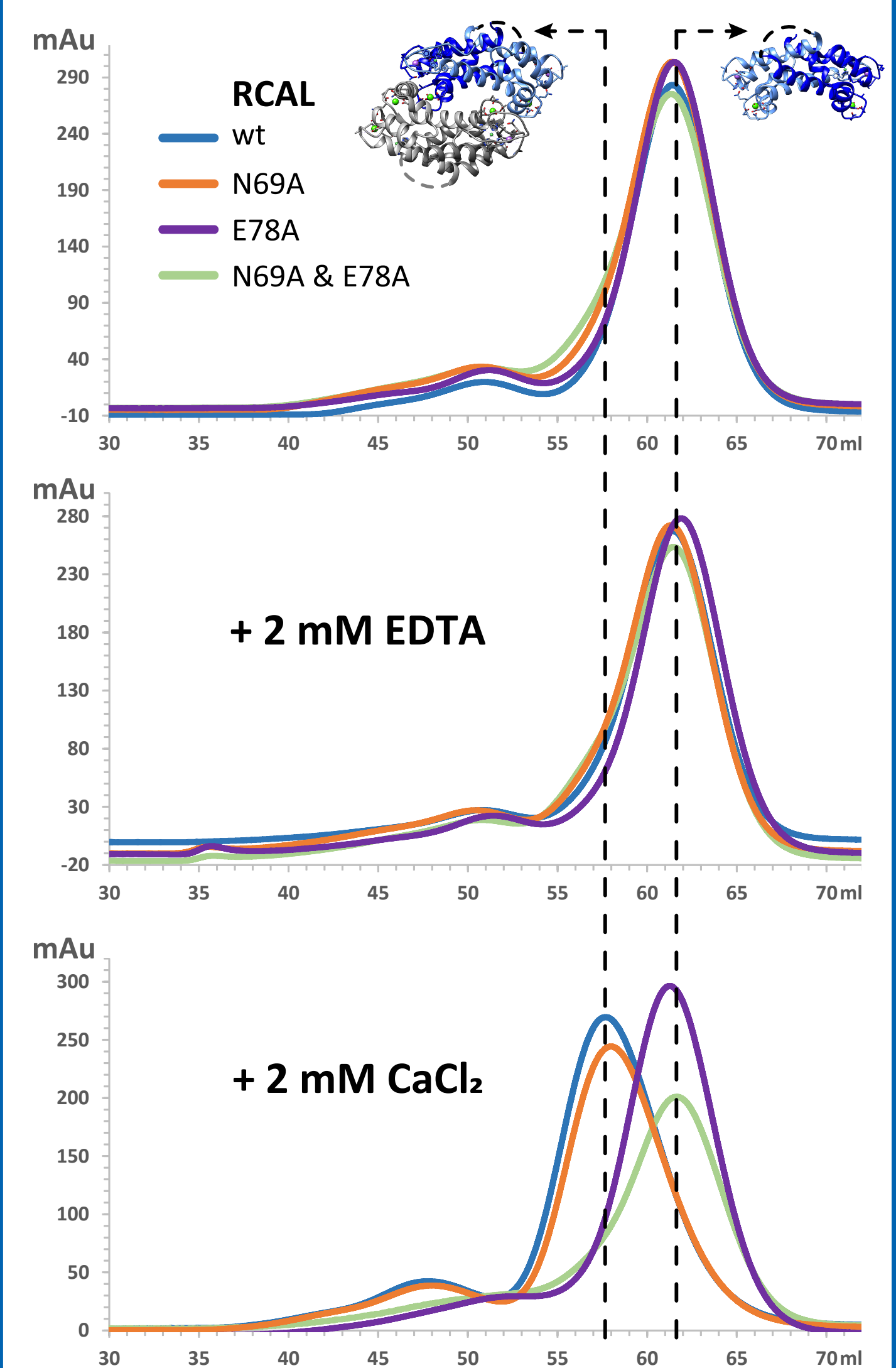


Depending on calprotectin's oligomeric state during calibration and in the patient sample, slopes between expected & measured calprotectin concentration can vary almost 8-fold.



Affinity measurements by BLI and SPR reveal similar affinities for native (NCAL, dark blue) and recombinant (RCAL, light blue) calprotectin, which are dependent on the oligomeric state for both.

Size exclusion analysis of RCAL

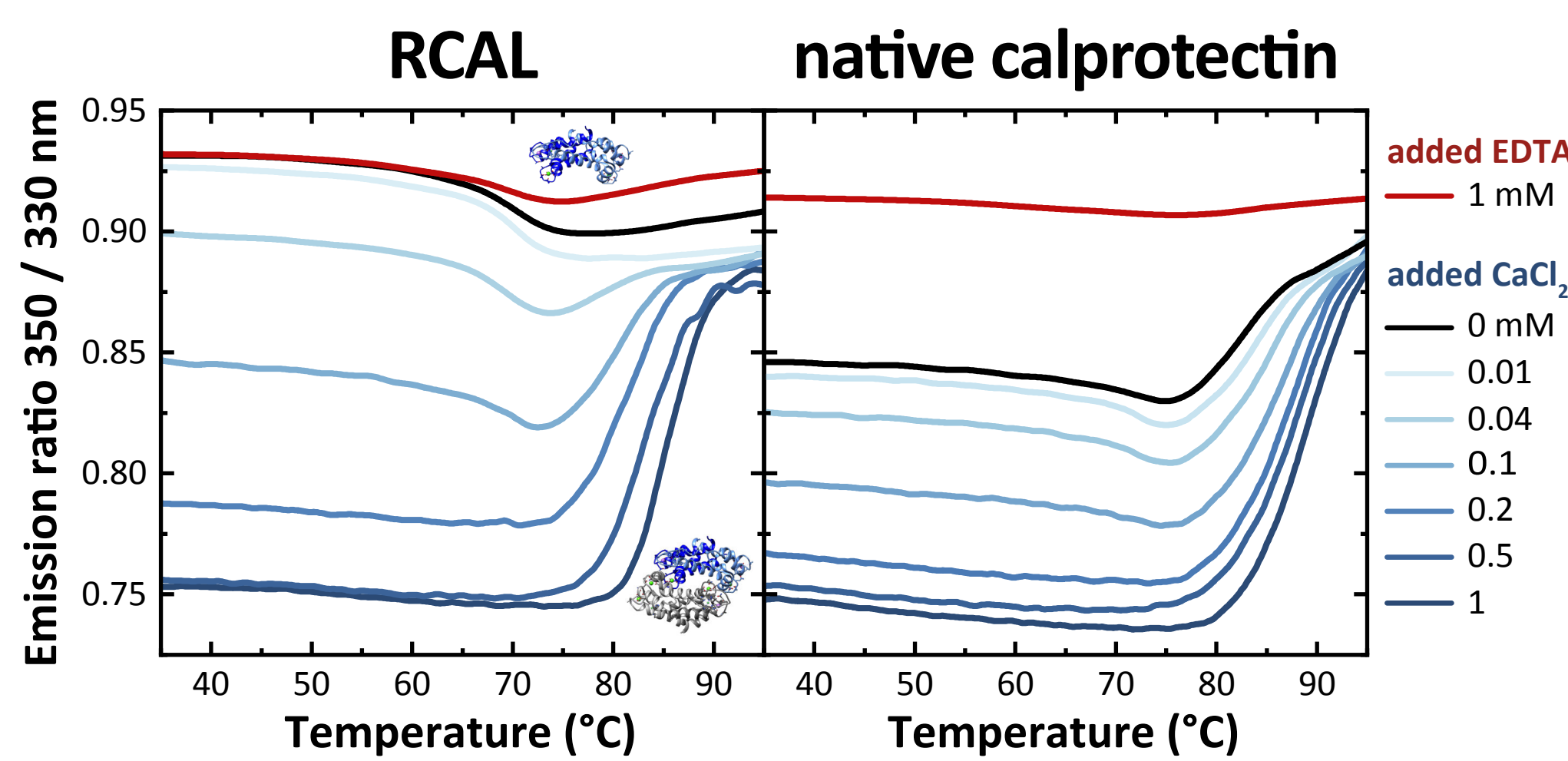


RCAL elutes at higher SEC volumes in the presence of 2 mM CaCl₂ indicating oligomerization. Mutations of the Ca²⁺ binding sites impair oligomerization in the case of MRP-14 E78A, but not N69A mutations. RCAL therefore mimics native calprotectin with respect to oligomerization.

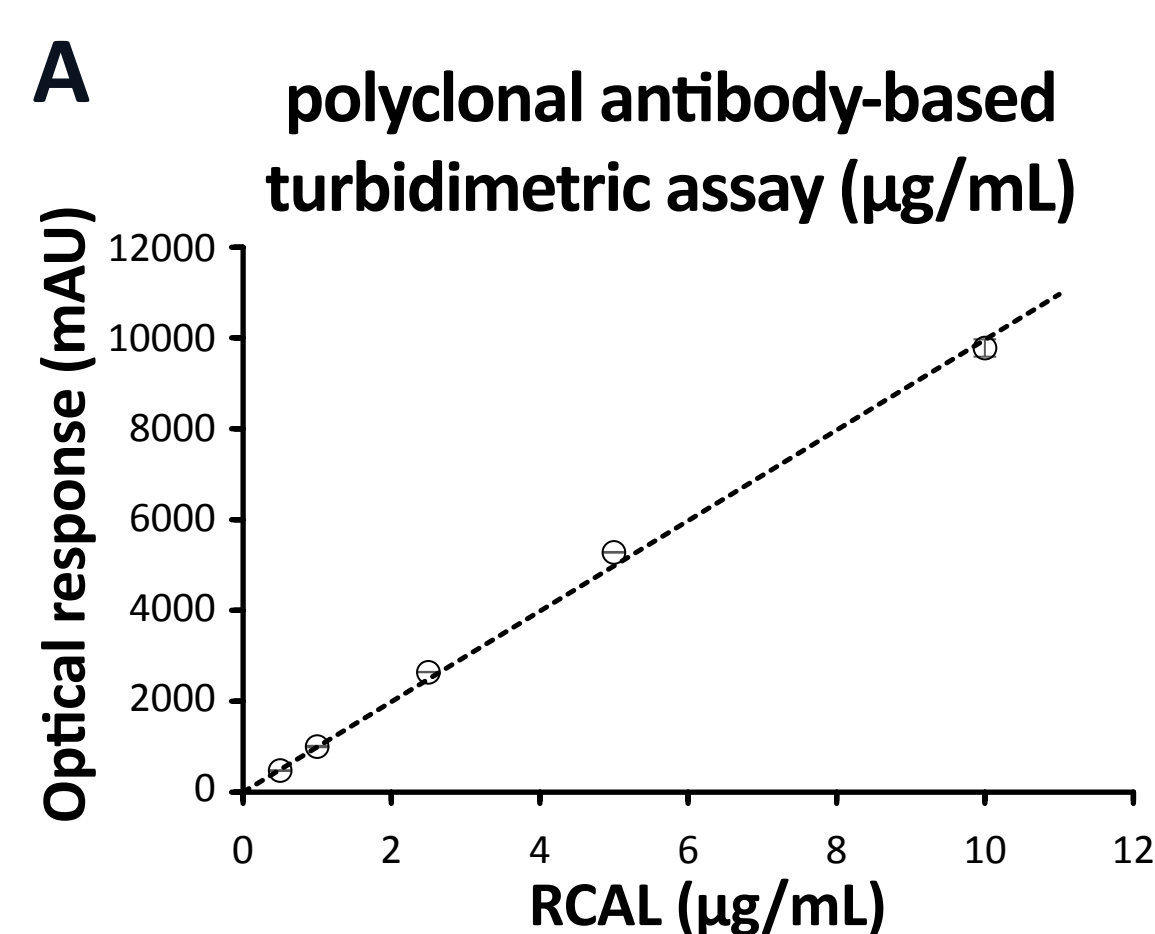
RCAL shows Ca²⁺-dependent transition comparable to native calprotectin

Label-free differential scanning fluorimetry reveals a transition upon CaCl₂ addition comparable to native calprotectin. Thermostability is comparable as well and increases with tetramerization.

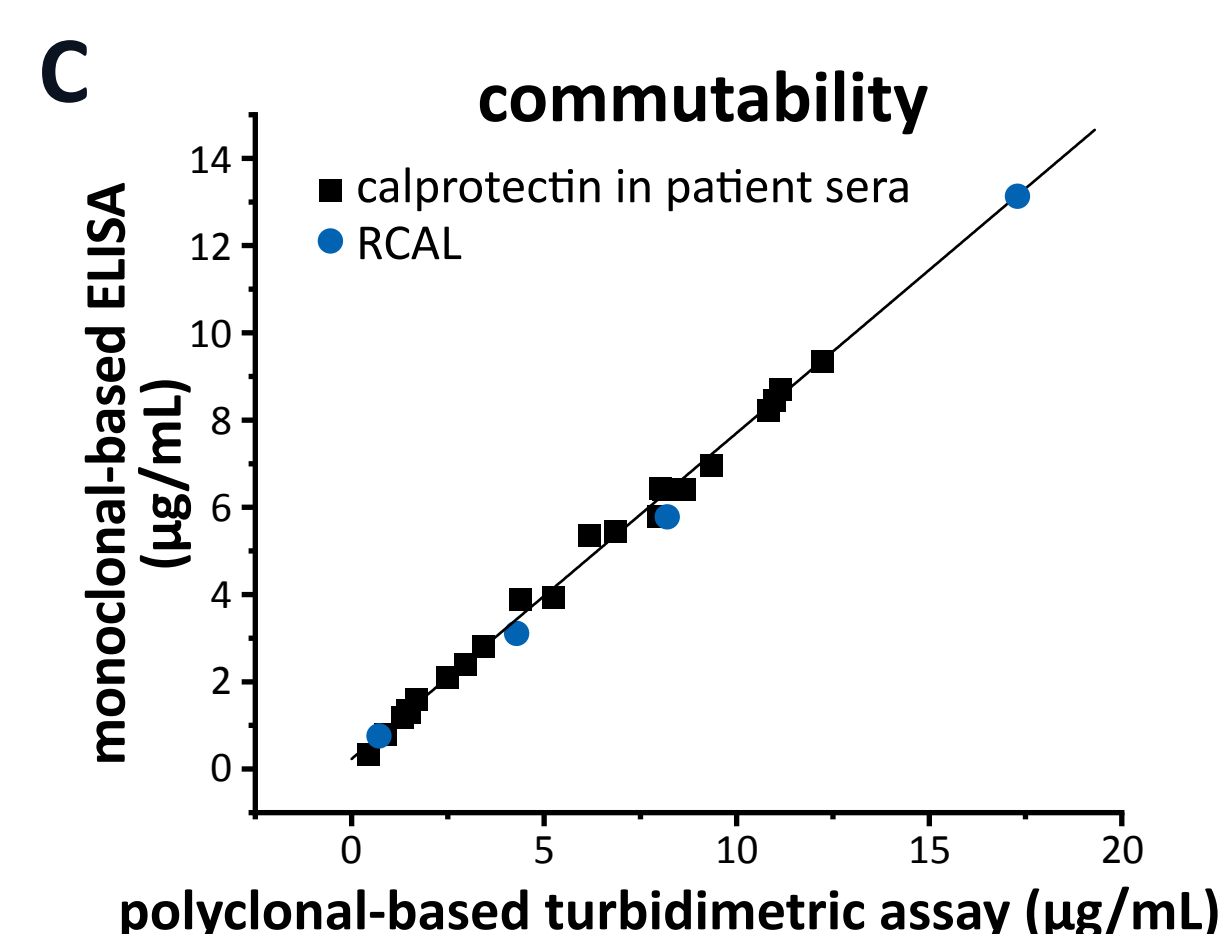
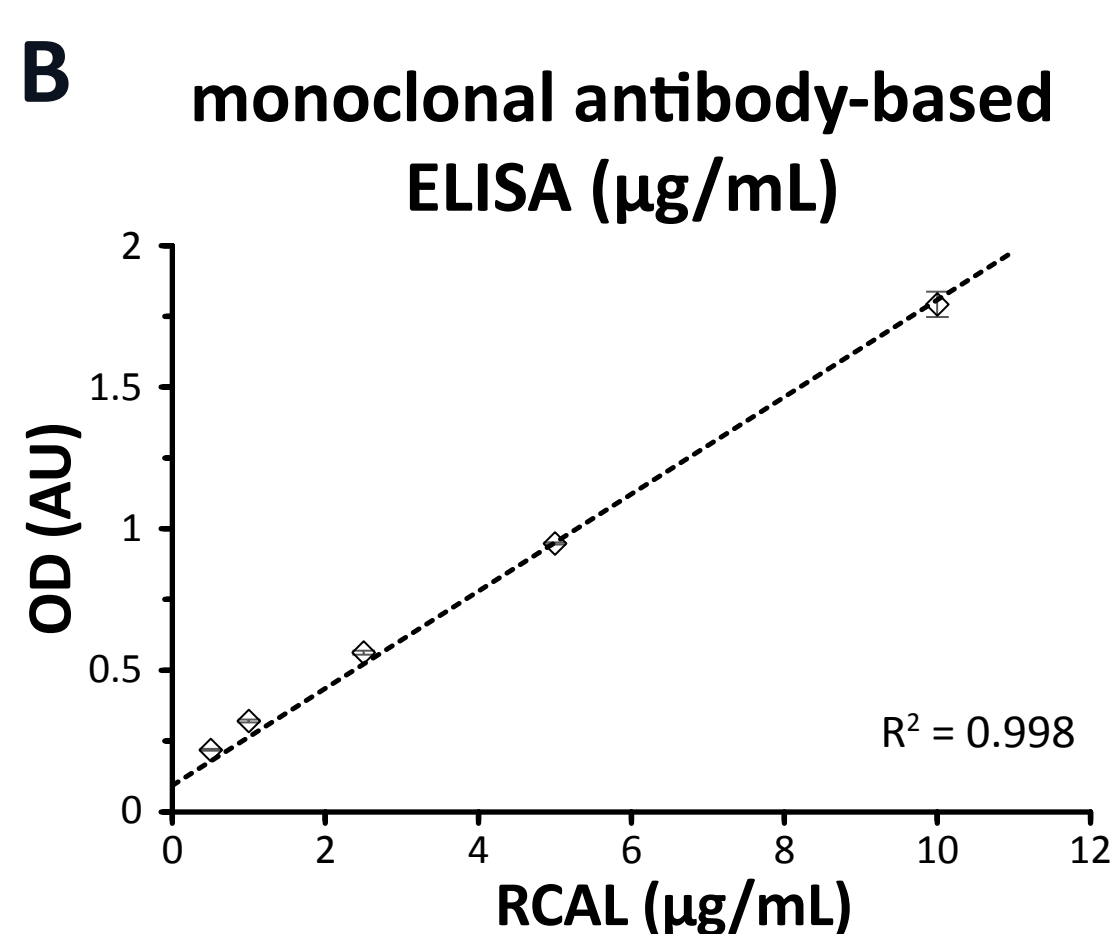
RCAL shows little change upon EDTA addition owing to the higher purity of the calprotectin dimer compared to native calprotectin.



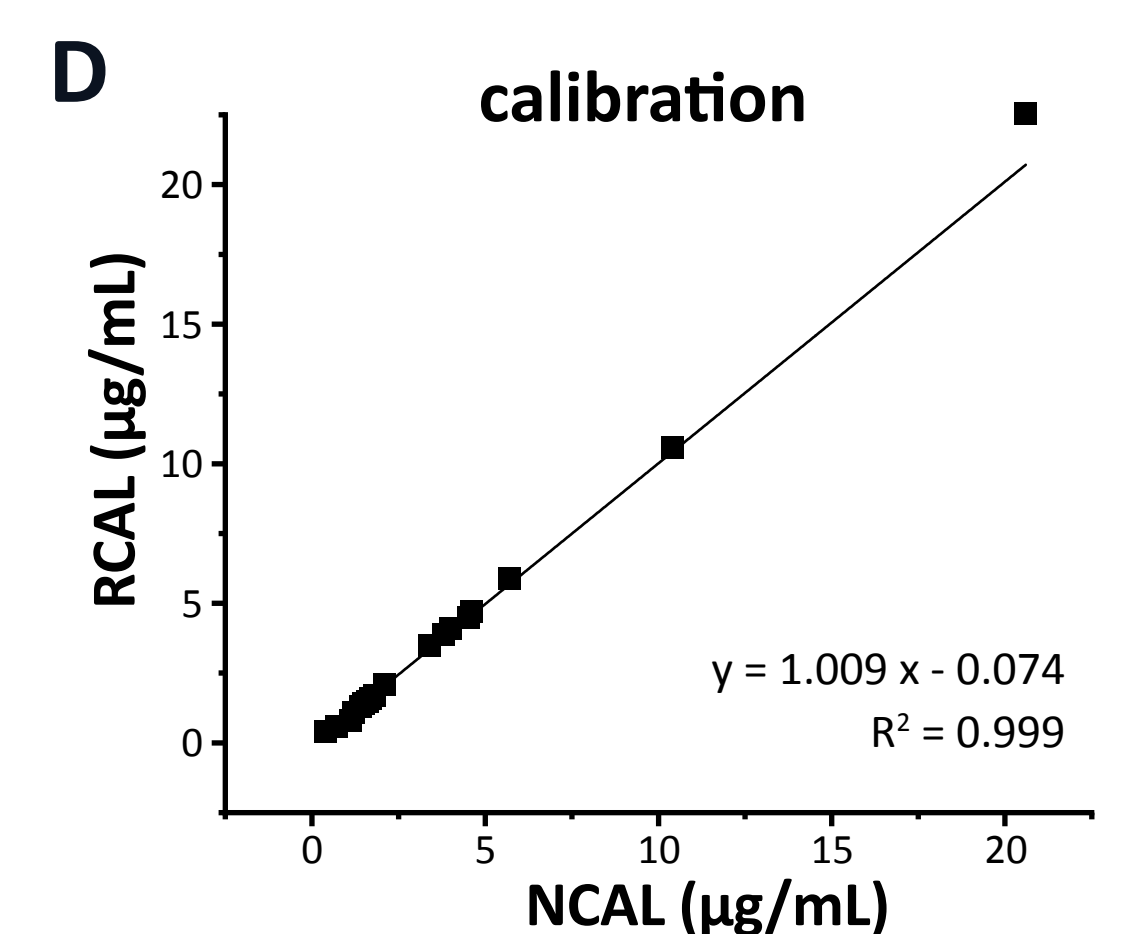
RCAL recognition in immunoassays



Concentration-dependent measurements of RCAL spiked incubation buffer shows linear behavior in the BÜHLMANN fCAL[®] turbo assay (A) and the fCAL[®] ELISA (B).

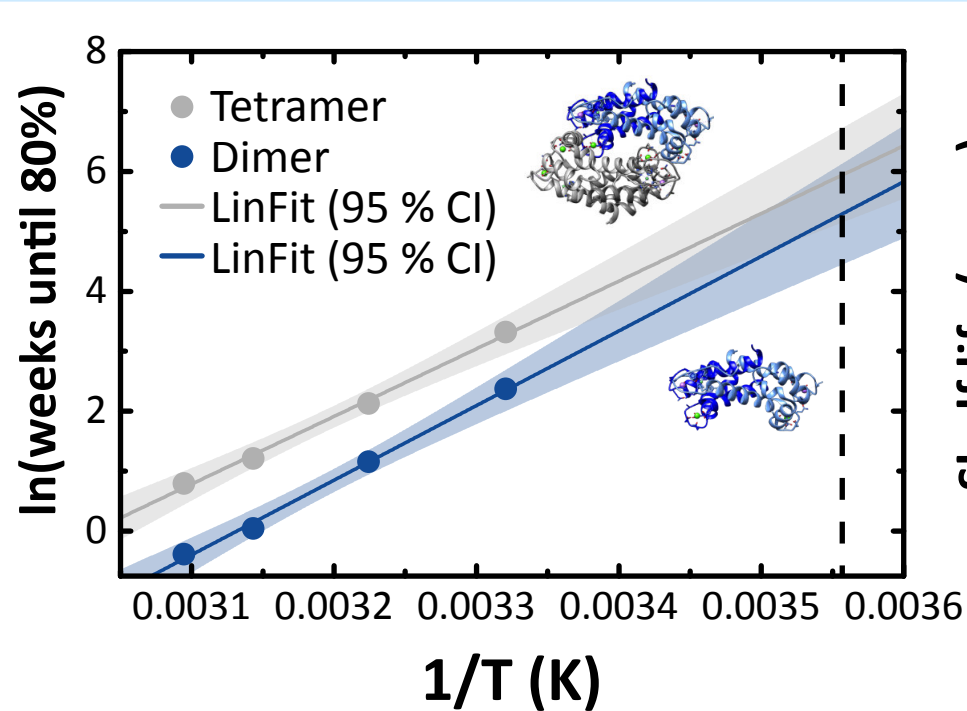


Measuring RCAL with a polyclonal antibody-based turbidimetric assay and a monoclonal antibody-based ELISA yields comparable data to 23 patient samples.



Measurements of 17 patient samples using fCAL[®] turbo based on calibration curves derived from RCAL or NCAL yield virtually identical values.

RCAL stability as a calibrator



Accelerated stability studies at higher temperatures allow shelf-life extrapolations for lower temperatures yielding >3 years for the dimer and >7 years for the tetramer at 8°C albeit with some uncertainty. Real-time studies are ongoing.

Key take-home messages

Our novel RCAL recombinant fusion calprotectin:

- shows immunological & biophysical properties comparable to NCAL (native)
 - can be purified in large quantities in defined oligomeric states
 - shows linear behavior in mono- and polyclonal immunoassays
 - yields similar results for patient samples if used as a calibrator instead of NCAL
 - exhibits sufficient stability to be used as calibrator material in IVD assays
- presents a promising tool to overcome the calprotectin standardization problem