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

Alexander Ohmann[1], Dmitrii Guschin[1], Joana Afonso[1], Peter Spies[2], Dominik Meinel[2], and Christian-B. Gerhold[1,*]

[1] BÜHLMANN Laboratories AG - 4124 Schönenbuch, Switzerland [2] Fachhochschule Nordwestschweiz FHNW - 4132 Muttenz, Switzerland

*correspondence to: cbg@buhlmannlabs.ch +41 61 487 12 52

BÜHLMANN n|w Fachhochschule Nordwestschweiz ueg week PP0662

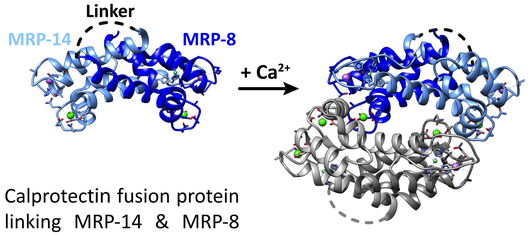
Motivation to produce recombinant calprotectin as a calibrator material

Calprotectin is a major granulocyte-derived alarmin protein that 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 yield different quantitative results yet trapping calprotectin in a distinct oligomeric state is challenging. It is therefore required to produce pure calprotectin as a calibrator material with a controllable oligomeric state.

Recombinant calprotectin RCAL



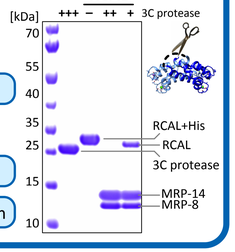
Calprotectin fusion protein linking MRP-14 & MRP-8 with a 3C-cleavable linker.

Calprotectin **dimer**

Calprotectin **tetramer**

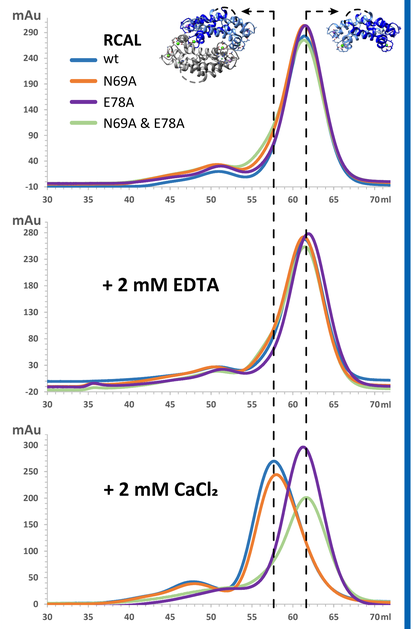
Expression & Purification

- Soluble bacterial expression
- Ni-NTA column
- *optional:* Anion exchange column
- Size exclusion chromatography
- *optional:* 3C protease cleavage
- *optional:* Ca^{2+}-induced oligomerization



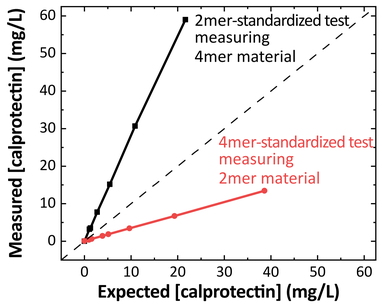
COI statement: Alexander Ohmann, Dmitrii Guschin, Joana Afonso and Christian-B. Gerhold are employees at BÜHLMANN Laboratories AG. Peter Spies and Dominik Meinel have no conflicts of interest to disclose.

Size exclusion analysis of RCAL

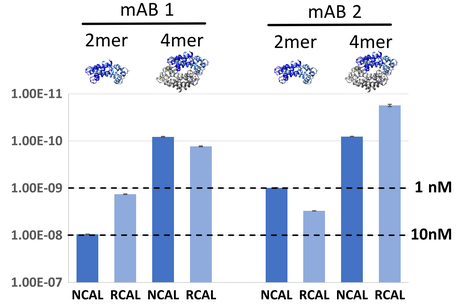


RCAL elutes at higher SEC volumes in the presence of 2 mM $CaCl_2$ indicating oligomerization. Mutations of the Ca^{2+} binding sites impair oligomerization in the case of MRP-14 E78A, but not N69A mutations. RCAL therefore mimics native calprotectin with respect to oligomerization.

Standardization & binding affinities depend on calprotectin's oligomeric state

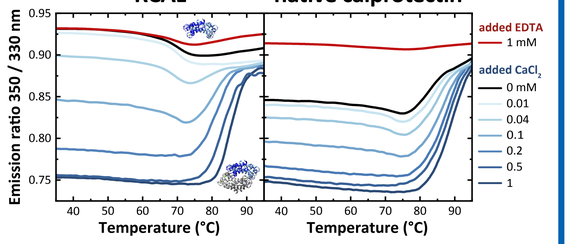


Depending on calprotectin's oligomeric state during calibration and in the patient sample, slopes between expected & measured calprotectin concentration can vary almost 8-fold.



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.

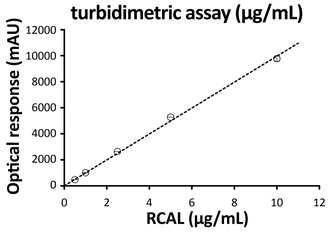
RCAL shows Ca^{2+}-dependent transition comparable to native calprotectin



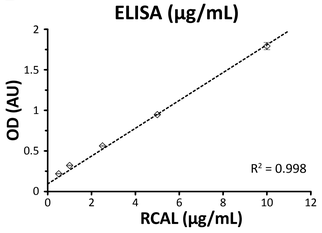
Label-free differential scanning fluorimetry reveals a transition upon $CaCl_2$ addition comparable to native calprotectin. Thermostability is comparable too and increases with tetramerization. RCAL shows little change upon EDTA addition due to the higher purity of the calprotectin dimer compared to native calprotectin.

RCAL recognition in immunoassays

A polyclonal antibody-based turbidimetric assay (µg/mL)

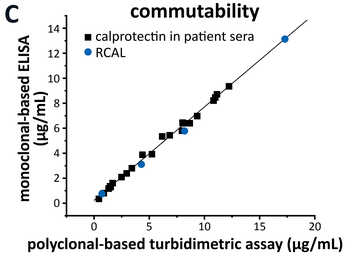


B monoclonal antibody-based ELISA (µg/mL)



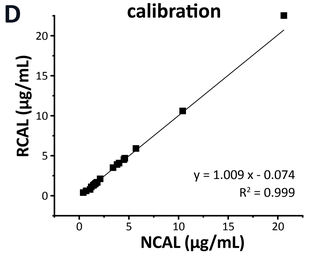
Concentration-dependent measurements of RCAL spiked incubation buffer shows linear behavior in the BÜHLMANN fCAL® turbo assay (A) and the fCAL® ELISA (B).

C commutability



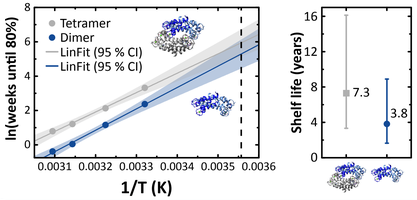
Measuring RCAL with a polyclonal antibody-based turbidimetric assay and a monoclonal antibody-based ELISA yields comparable data to 23 patient samples.

D calibration



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-live extrapolations 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**