Supplementary Material (ESI) for Soft Matter


# Electronic Supplemental Information

## Formation and stability kinetics of calcium phosphate-Fetuin-A colloidal particles probed by time-resolved dynamic light scattering

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### CPP size analysis

Mineral sedimentation easily occurs in solutions that are supersaturated with regard to calcium and phosphate ions. The serum protein fetuin-A is able to inhibit this process transiently. It has been shown previously that the inhibition is associated with the formation of fetuin-A – mineral colloids, denoted as calciprotein particles (CPP). After a lag period initial (primary) CPPs (CPP1) rapidly transform to larger secondary CPPs (CPP2). We termed this process "ripening" in analogy to Ostwald ripening.
The data of all time-resolved DLS measurements are compiled in Table S1. Figure S1 represents a graphical illustration of the CPP sizes. Under low and high mineral ion supersaturation (LS, HS; see methods) conditions, an increase in fetuin-A concentration led to smaller primary and secondary CPPs. Moreover, LS conditions produced slightly larger particles than high mineral ion supersaturation (HS) conditions. Under LS conditions, the two-way analysis of variance (ANOVA) also revealed a statistically significant fetuin conc. – temperature interaction regarding CPP1 size. Regarding CPP2, the statistical analysis revealed in addition to the fetuin-A effect that higher temperatures resulted in larger particles. Under HS conditions, no temperature effect was found. Hence in this case, CPP ripening is apparently governed by mineral ion supersaturation, outweighing the temperature effect.

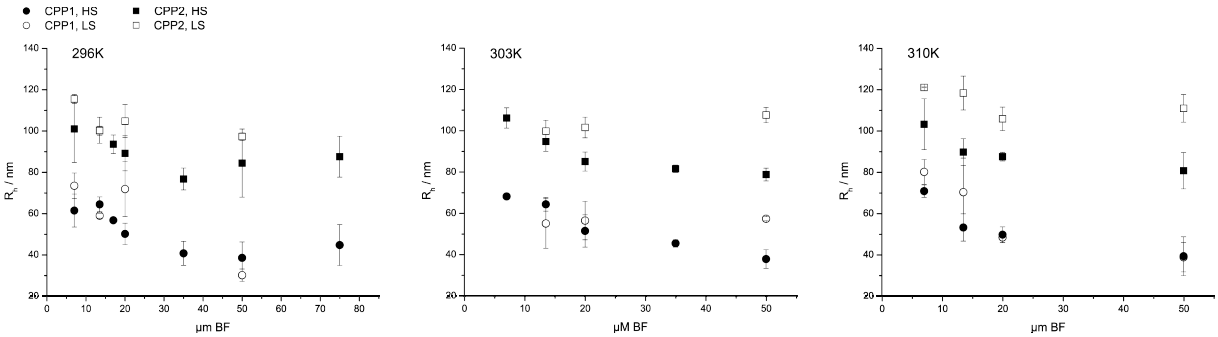


**Figure S1:** Bovine fetuin-A (BF) concentration dependent plot of CPP sizes at three temperatures and two mineral ion concentrations. HS indicates high supersaturation conditions: 10 mM $CaCl_2$ und 6 mM $Na_2HPO_4$, LS indicates low supersaturation conditions: 5 mM $CaCl_2$ und 3 mM $Na_2HPO_4$. The hydrodynamic radii of primary CPPs (CPP1) range from 40 to 70 nm whereas the secondary CPPs (CPP2) range from 80 to 120 nm.

**DLS data**

| | $R_h$ (CPP1) / nm | | | $R_h$ (CPP2) / nm | | | $t_{trans}$ / min | | |
|---|---|---|---|---|---|---|---|---|---|
| **LS** | | | | | | | | | |
| T / K | 296 | 303 | 310 | 296 | 303 | 310 | 296 | 303 | 310 |
| BF / µM | | | | | | | | | |
| 7 | 73.5±6.2 | | 80.2±6.0 | 115.5±2.2 | | 121.1±0.2 | 717±151 | | 75±6 |
| 13.5 | 59±1.2 | 55.1±12.3 | 70.4±16.5 | 102.6±1.7 | 99.8±5.3 | 118.4±8.2 | 778±130 | 471±67 | 138±39 |
| 20 | 68.8±12.5 | 56.5±9.3 | 48.5±2.6 | 103.1±7.3 | 101.6±5 | 105.9±5.7 | 720±67 | 420.±226 | 174±23 |
| 50 | 30.2±3 | 50.2±12.3 | 38.9±7.1 | 97±1.8 | 108.7±3.3 | 111±6.6 | 1111±51 | 463±33 | 200±42 |
| **HS** | | | | | | | | | |
| T / K | 296 | 303 | 310 | 296 | 303 | 310 | 296 | 303 | 310 |
| BF / µM | | | | | | | | | |
| 7 | 61.5±8 (63±6) | 68.17±1.2 | 70.9±3 | 101±16.2 (103±13) | 106.17±4.9 | 103.3±12.3 | 332±23 | 118±15 | 44±12 |
| 13.5 | 64.5±3.6 | 64.4±3.3 | 53.3±6.6 | 100.4±6.3 | 94.8±4.7 | 89.8±6.5 | 455±5 | 180 | 55±11 |
| 20 | 50.2±5.2 (44±6) | 51.5±7.8 | 49.8±3.8 | 89.2±5.3 (80±5) | 85.1±4.6 | 87.6±2.1 | 396±47 | 178±22 | 60±12 |
| 35 | 40.8±5.8 | 45.5±1.8 | 46.7±6.8 | 76.8±5.3 | 81.6±1.8 | 86.0±2.7 | 595±103 | 256±42 | 78±15 |
| 50 | 38.6±7.7 (38±9) | 37.9±4.5 | 39.4±9.4 | 84.5±16.5 (72±8) | 78.8±3.1 | 80.8±8.8 | 475±34 | 202±3 | 73±2 |

**Table S1:** comprehensive presentation of the data obtained from DLS measurements. For comparison, the radii in brackets were taken from ref. 23. For each group, i.e. LS - HS conditions, CPP1 - CPP2, we performed a two-way analysis of variance (ANOVA using Graphpad Prism Software) of the hydrodynamic radius $R_h$ with a significance level of $p < 0.05$. The effects of fetuin-A concentration, temperature and mineral ion supersaturation are compiled in table 3.

**Abbreviations:**

| | |
|---|---|
| Bovine fetuin-A | BF |
| Calciprotein particles | CPP |
| Dynamic light scattering | DLS |
| High mineral ion supersaturation | HS |
| Low mineral ion supersaturation | LS |