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# Comparison of optomagnetic and AC susceptibility readouts in a magnetic nanoparticle agglutination assay for detection of C-reactive protein

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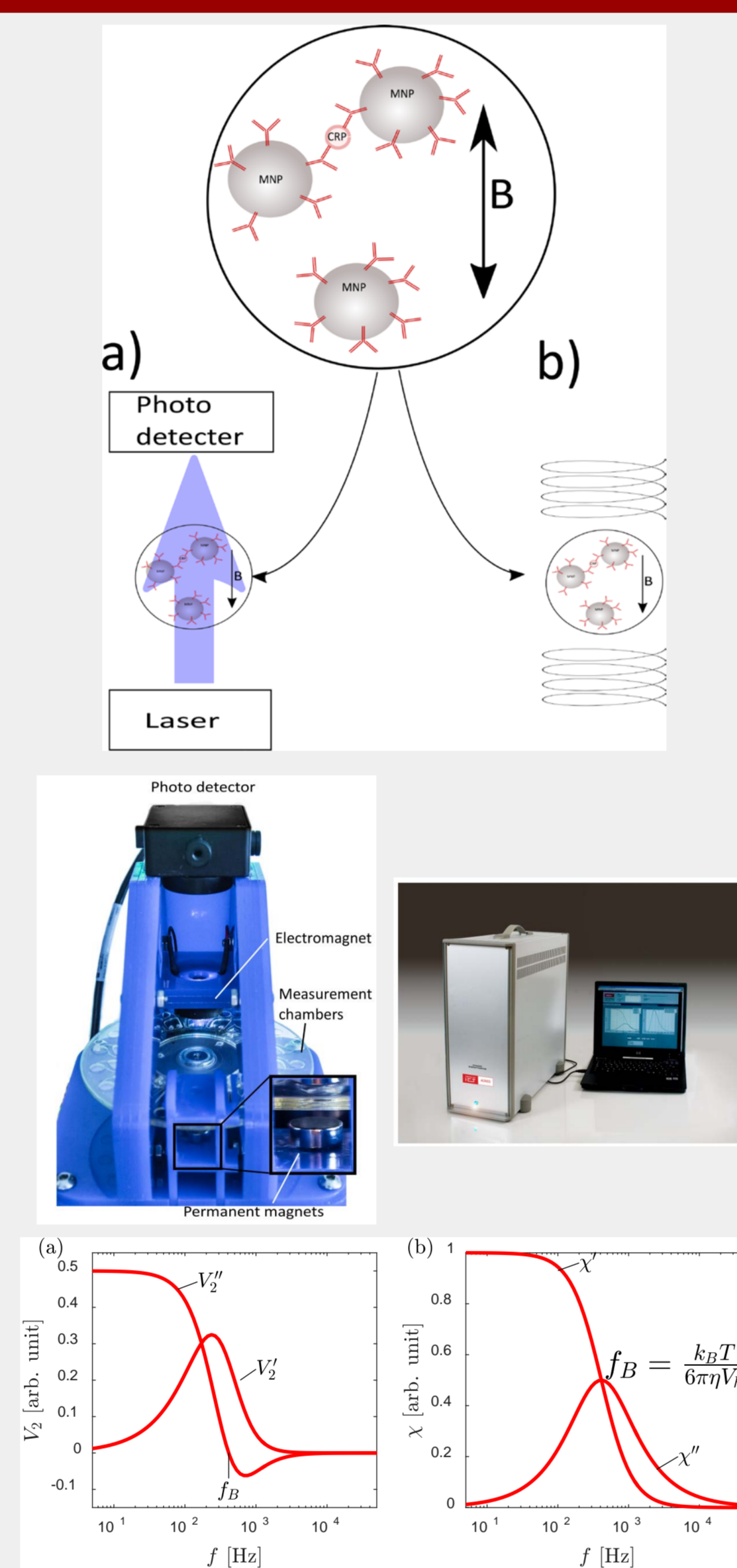
## Abstract & Method

C-reactive protein (CRP) is an important marker for inflammation. We present for the first time a comparison between readouts of the dynamic response of a Magnetic nanoparticle (MNP) suspension vs. concentration of a CRP target using an AC susceptometer (magnetic signal) and a recently proposed optomagnetic technique (optical signal) [1].

The agglutination assay consists of:

1. MNPs functionalized with CRP antibodies
2. CRP which results in links between MNPs
3. An oscillating magnetic field
4. A readout of Brownian rotation:
  - a) Optical (Laser and photo-detector)
  - b) Magnetic (pickup coils)

In an oscillating magnetic field, clusters of MNPs are not able to rotate as fast as individual MNPs and thus contribute to the signal at lower frequencies.



## Results

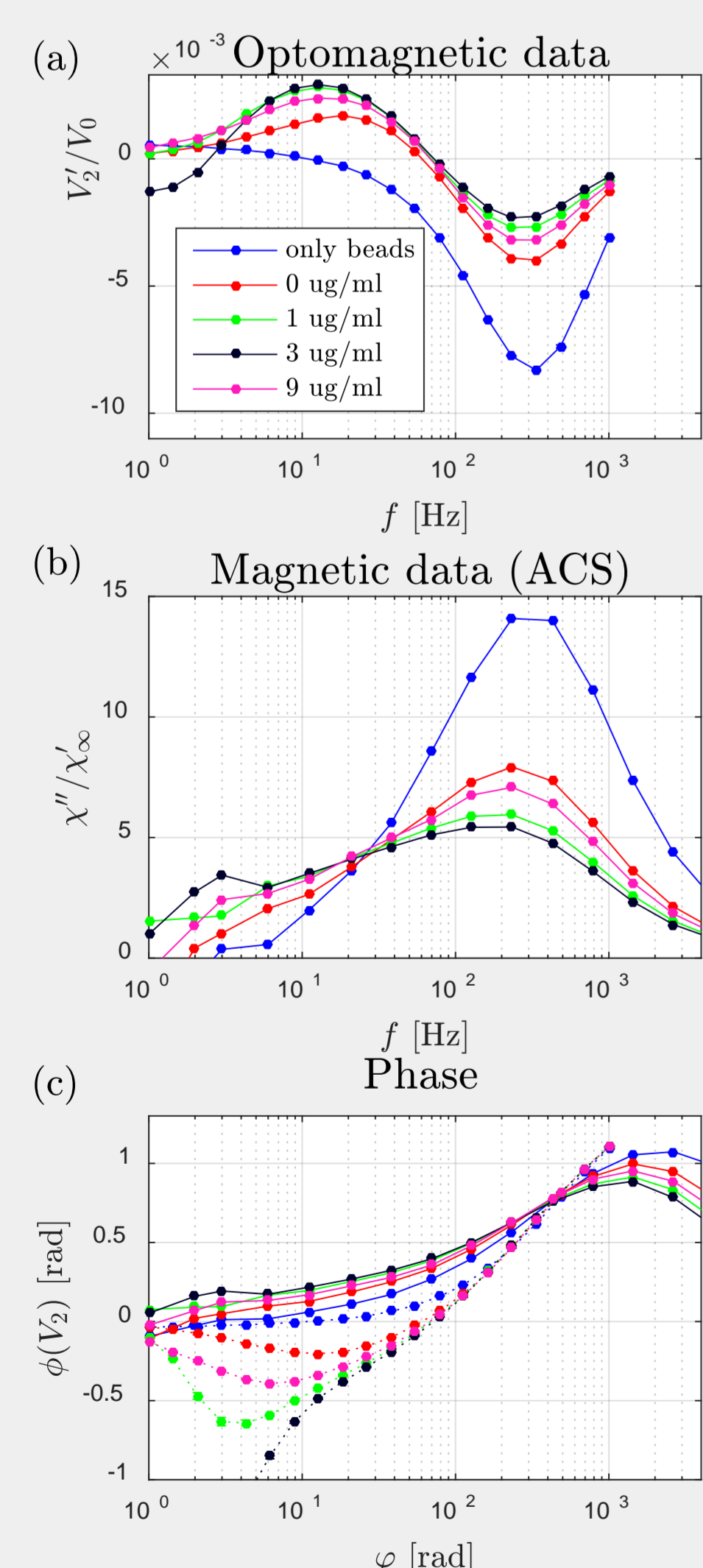


Fig. 1. Data of agglutination assay for functionalized beads in the presence of buffer (Blue) and <0.02 (red), 1 (green), 3 (black), 9 (pink)  $\mu\text{g/ml}$  CRP. (a) Real part of the second harmonic optomagnetic signal normalized with the total light intensity. (b) Imaginary part of the AC susceptibility normalized with the infinity frequency limit of the real part,  $\chi''_{\infty}$ . (c) Magnetic phases of optomagnetic data (dotted lines) and ACS data (solid lines)

- Functionalized beads without added serum (blue) only give signals from single MNPs:
  - (a) optomagnetic data at  $\sim 340$  Hz
  - (b) ACS data at  $\sim 250$  Hz.
- In CRP-free serum ( $<0.02 \mu\text{g/ml}$ ) the single MNP signal decreases and a signal at lower frequency ( $<60$  Hz) increases (red curves) indicating MNP agglomeration.
- Agglomerates give rise to:
  - (a) peak with opposite sign to the free MNP signal for the optomagnetic data.
  - (b) shoulder to the free MNP signal for the magnetic data (ACS).
- Increasing the CRP concentration (green and black curves) increases agglomeration.
- For the highest CRP concentration ( $9 \mu\text{g/ml}$ ) aggregation is reduced (purple curves). This “hook effect” arises when the target saturates the CRP binding sites and prevents agglomeration.

## Dose response

### (a) Turn-off detection:

- Measures the depletion of free MNPs at  $\sim 300$  Hz
- High correlation ( $r = 0.999$ ) between ACS and optomagnetic method

### (b) Turn-on detection:

- Measures the formation of aggregates at 4 Hz (+) and 12 Hz (x).
- ACS low-frequency data are noisy because of inductive readout.
- Optomagnetic signal displays several sign changes at low frequencies.

### (c) Phase detection

- Signal phase at 4 Hz (+) and 12 Hz (x).
- The phase change for the:
  - magnetic signal is almost independent on the frequencies (for  $f < 100$  Hz).
  - optomagnetic signal depends on  $f$ .
- At 12 Hz the phases correlate very well ( $r = 0.999$ ).

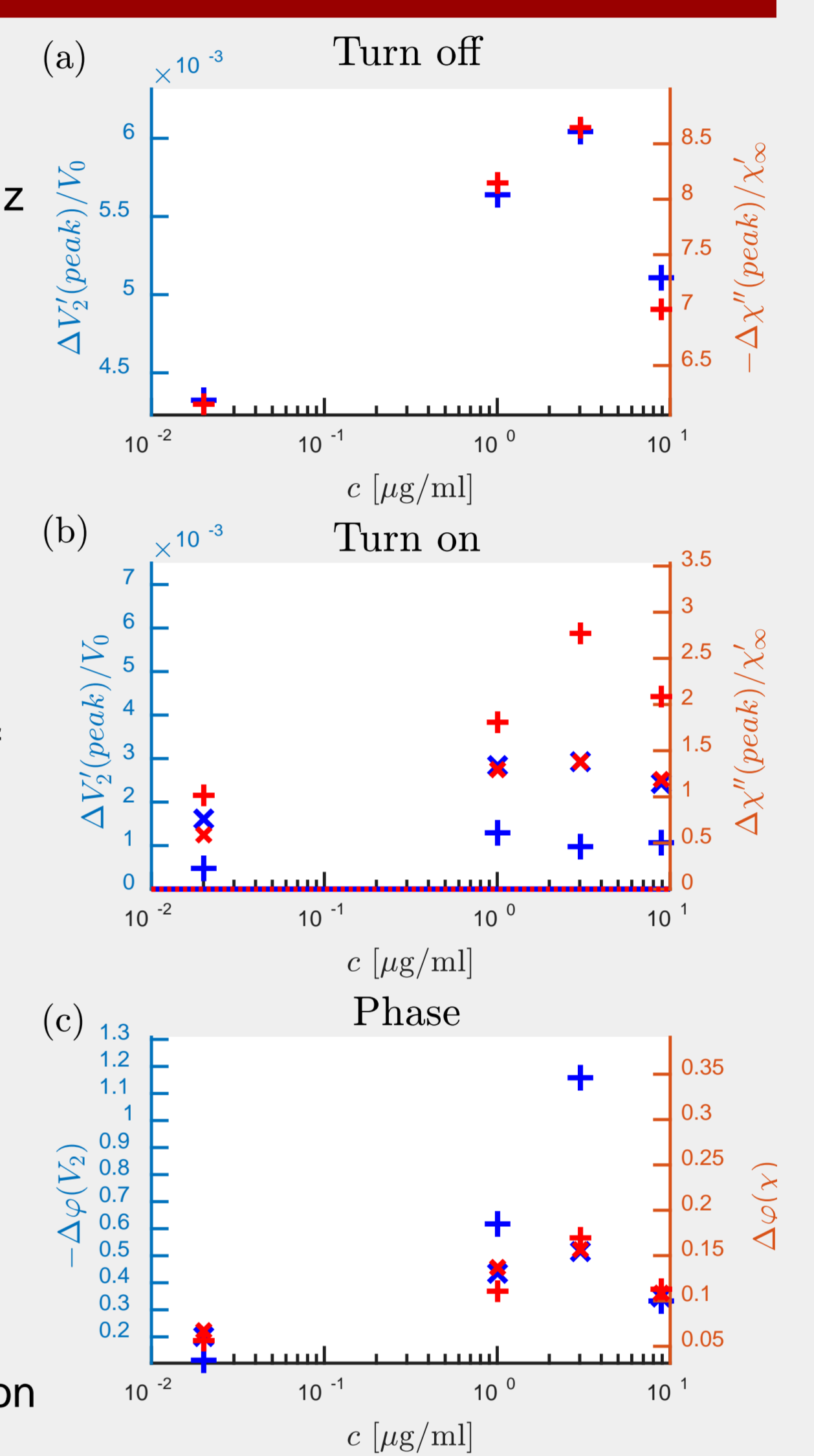


Fig 2. Dose-response curve constructed from optomagnetic data (blue, left y-axis) and AC susceptibility data (red, right y-axis). The differences to the values for the beads without serum is shown.

## Discussion & Conclusion

- High correlation of *turn-off* detection strategy shows that when measuring the depletion of signal of only one species, the methods provide the same results.
- Lower correlation of *turn-on* method is due to signal arises from a distribution of aggregates, which have different weighting in the two methods. The two sign changes of the optomagnetic signal and the noisy ACS data make this method a nonviable strategy.
- Phase signal cancels variations in bead concentration, and have previously been shown to give a very reproducible signal for optomagnetic detection.
- The sign change of the optomagnetic signal makes the signal difficult to interpret. However, it makes it very sensitive to formation of aggregates.

## Acknowledgements

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