COMPASS – rapid and highly sensitive medical point-of-care diagnostic

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Abstract

In the last decade Magnetic nanoparticles (MNPs) have gained an enormous interest in specialized areas such as medicine, cancer theranostics, biosensing, catalysis, agriculture, and the environmental protection. By controlled engineering of specific surface properties, named functionalization, MNPs are gaining special features for desired applications, e.g., bioassays for the detection of biomolecules or biomarkers such as antibodies.

The characterization as well as a highly specific measurement of such binding states is of high interest and limited to highly sensitive techniques such as ELISA (Enzyme-linked Immunosorbent Assay) or flow cytometry, which are relatively inflexible, difficult to handle, expensive and time-consuming.

Novel upcoming methods, such as ACS (AC susceptometry) or MPS (Magnetic Particle Spectroscopy), exploit the magnetization response of functionalized MNP ensembles to assess specific information about the MNP mobility within their environment as well as the conjugations of chemical or biological compounds on their surface. Both methods have shown promising results reaching similar sensitivities within short measurement times but showing difficulties in data interpretation.

Here, we report a novel method, COMPASS (Critical Offset Magnetic Particle Spectroscopy), which is based on a critical offset magnetic field of MNPs, which enables sensitive detection to minimal changes in mobility of MNP ensembles, e.g., resulting from SARS-CoV-2 antibodies binding to the S antigen on the surface of functionalized MNPs. With a validated sensitivity of 0.85 fmole/50 µl sample volume (~33 pM) SARS-CoV-2-S1 antibodies, measured with a low-cost portable COMPASS device, the proposed technique is not only competitive with the sensitivity of commonly used ELISA or flow cytometry methods but provides more flexibility, robustness and rapid measurement within well below a minute per sample, including sample conjugation, mixing and incubation times.

The underlying physical effect is based on an offset magnetic field induced suppression of a higher harmonic in the nonlinear magnetization response of the MNP to a time varying magnetic field resulting in a highly sensitive response of the signal phase to minimal changes in particle mobility. Since this effect is independent of MNP concentration, the sample handling is much simpler and robust.

Our method thus may pave the way for deeper insights into complex and rapid binding dynamics of functionalization chemistry and can lead to a huge step forwards in point-of-care diagnostics as well as impacts other fields in research and industries.

Full Text

The characterization of ensembles of magnetic nanoparticles (MNP) is a dynamically developing field and found various applications in many fields of research such as medicine, cancer theranostics, biosensing, catalysis, agriculture, and the environment [1-3]. Thus, a huge portfolio of different methods and techniques is available today to investigate the complex dynamics of MNP ensembles [4, 5].
Magnetic particle spectroscopy (MPS) is a quite young technology for the characterization of MNPs. It uses an oscillating magnetic field of sufficient field strength to drive the MNP ensemble periodically into their non-linear magnetization response [6]. This reveals specific information for each MNP type in form of higher harmonics of the excitation frequency and can be used to measure parameters such as hydrodynamic diameter or viscosity and temperature of the surrounding solution as well as the conjugations of chemical or biological compounds on the surface of the MNPs. In short, MPS is able to investigate the mobility of MNPs [7].

Due to the fact, that MPS directly measures the analytical signals from the entire sample volume is making bioassays simple and fast [8-10]. E.g., functionalization of the surface of the MNPs by anchoring linkers, such as specific antibodies, allows detecting viral proteins by binding specific epitopes. Cross-linking between the MNPs influences their mobility resulting in a minimal signal change. This offers the detection of, e.g., 44 nM H1N1 nucleoprotein or 1.56 nM SARS-CoV-2 spike protein within a measurement time of about 10 seconds [8, 10]. However, since the sensitivity of MPS is mainly based on the particle core composition and not on the environmental serum, the signal change in the experiments between binding and non-binding samples is quite small. In addition, the signal as well as its change strongly depends on MNP and the analyte concentration, which requires sophisticated sample handling and data processing to robustly detect the relevant signal changes.

Similar modalities using MNPs, such as AC-susceptometry (ACS) measurement [11-14] provide a more sensitive technique to investigate and determine parameters of the environmental serum, e.g., rapid detection of 84 pM mimic SARS-CoV-2 in 36 s [11]. But ACS is complicated by long acquisition times, the complexity of handling of those devices as well as the data processing, which require sophisticated hardware and experienced personnel.

Common MPS devices are working with a strong time varying magnetic field $H_{AC}$, while ACS devices are working with weak excitation fields $H_{AC}$ below 2 mT and multiple frequencies $f_{AC}$ and sometimes with additional strong offset magnetic fields $H_{DC}$ (static or with low frequency $\ll f_{AC}$).

We combined a strong excitation field $H_{AC}$ with a strong offset magnetic field $H_{DC}$ and expand the parameter space with COMPASS (Critical Offset Magnetic Particle Spectroscopy) as indicated in Fig. 1. This allowed extremely sensitive and robust investigation of MNP dynamics and surface chemistry at critical offset fields which to our knowledge was not exploited before and allows for measurements with higher sensitivities than MPS or ACS. Furthermore, COMPASS reaches a detection limit of SARS-CoV-2-S1 antibodies binding to the S antigen on a functionalized surface of MNPs, which is comparable with the gold-standard methods ELISA (Enzyme-linked Immunosorbent Assay) [15] and flow cytometry [16]. While both techniques are limited by their inflexibility, the complex handling, and the long measurement time, COMPASS provides a robust and easy-to-use testing environment.

**Physical background of critical points**
The magnetization of a superparamagnetic sample depends on the surrounding magnetic field $H = H_{AC} + H_{DC}$ consisting of dynamic $H_{AC}$ and static $H_{DC}$ magnetic fields. Particles, which usually exhibiting superparamagnetic properties, consist of a single magnetic domain and can be seen as tiny permanent magnets (single domain particles). In absence of an external magnetic field, all nanoparticles of such an ensemble (sample) are statistically oriented, which causes the magnetization of the sample to be zero.

Increasing the external magnetic field strength leads to more and more particles aligning along the external magnetic field resulting in an increase of the magnetization. At a specific magnetic field strength $M_{sat}$, all particles are aligned and the magnetization of the sample is saturated (saturation magnetization $M_{sat}$). The dependency of the sample magnetization $M$ on the external magnetic field strength $H$ can be described by the Langevin function $L(\xi)$:

$$L(\xi) = \coth(\xi) - \frac{1}{\xi} \quad \text{with} \quad \xi = \frac{\mu_0 m H}{k_B T},$$

with $m$ as the magnetic moment of a particle, $\mu_0$ as the vacuum permeability, $k_B$ as the Boltzmann constant and $T$ as temperature. The Langevin parameter $\xi$ describes the different regimes of the magnetization response: $|\xi| \ll 1$ describes the linear regime for small external magnetic fields and $|\xi| \geq 1$ describes the non-linear regime (Fig. 2 a). However, it is important to note that Eq. (1) is only an approximation to real particles. Especially the assumption that the magnetization follows the external field instantaneous is not fulfilled (see supplementary S2).

MPS devices are using time-varying magnetic excitation fields $H_{AC}(t) = H_0 \cdot \sin(2\pi \cdot f_1 \cdot t)$, which are sufficiently high to drive the magnetization $M$ of a sample periodically with frequency $f_1$ into their nonlinear response. In contrast, the magnetic field strength of ACS devices is much smaller ($H_{0,ACS} < 2 \text{ mT} < H_{0,MPS}$). That means, ACS investigates the behavior of the sample in the linear regime ($|\xi| \ll 1$) determining the susceptibility or slope ($\chi = dM/dH$) of the magnetization curve while MPS is more focused on the non-linear response of the magnetization ($|\xi| \geq 1$).

The magnetization response $M(t)$ over time of a sample during continuous magnetic field excitation $H_{AC}(t)$ larger than 5 mT (MPS) approximates a mostly rectangular shape depending on the amplitude $H_0$ of the excitation field. An analysis of the time signal using a Fourier transformation reveals odd higher harmonics $(2n-1) \cdot f_1$ ($n \in \mathbb{N}$) of the excitation frequency $f_1$ in the spectrum due to the symmetric behavior of the signal over one period $1/f_1$. These higher harmonics are specific for the MNP type and encode information of its magnetic response and, hence, on the properties of the particle or its surrounding.

During ACS experiments, only the fundamental frequency $f_1$ is usually studied at different frequencies in the linear regime to get a frequency-dependent characterization of the MNPs [11-14].

For both ACS and MPS the application of static offset magnetic field $H_{DC}$ parallel to the excitation field $(H_{AC}(t) \parallel H_{DC})$ extends both methods and allows for a closer investigation of the magnetization curve in...
the non-linear regime.

During MPS experiments in the presence of an offset magnetic field $H_{\text{DC}}$ the magnetization response $M(t)$ becomes asymmetric, which introduces higher even harmonics $2n f_1$ ($n \in \mathbb{N}$) of the excitation frequency $f_1$ in the Fourier spectrum (Fig. 2 a&b).

Investigating the spectral components $A_n$ of each higher harmonic $n$ in dependence of the offset magnetic field strengths $H_{\text{DC}}$, the real and imaginary part of $A_n(H_{\text{DC}})$ show an interesting behavior. For $H_{\text{DC}} < H_{\text{AC}}$ a wavelike functional dependence on $H_{\text{DC}}$ with zeroes, also called nodes, at offset fields specific for each harmonic $n$ is observed (Fig. 2 c&d) \textit{(see gif-animations)}. This behavior can be described by a convolution of Chebyshev polynomials of second kind $U_n$ with the derivative of the magnetization curve $M'=dM/dH$ \textit{(see supplementary S1)} \cite{17}. With increasing harmonic number $n$, the spectral component $A_n(H_{\text{DC}})$ shows an increasing number of nodes. The corresponding phase plot $\phi_n(H_{\text{DC}})$ of the harmonic signal shows a steep slope of the phase near such nodes or ‘dips’. Hence, minimal changes in the magnetization response curve due to changes in particle or environmental parameters, e.g., hydrodynamic diameter, lead to a strong detectable phase difference $d\phi=\phi_{\text{res}}=\phi_1-\phi_2$ between two experiments with two different samples \textit{(see supplementary S2)}. This implies a high sensitivity on changes of the sample parameters at these distinct offset field induced nodes which are, hence, called critical points (CPs) in the following.

\textbf{Critical points sensitivity evaluation}

To evaluate the novel COMPASS method, we hypothesized that we can exploit COMPASS to detect SARS-2 specific antibodies as these bind to MNP ensembles with sensitivities competing with ELISA and flow cytometry. Furthermore, decreasing the serum conjugation time down to several seconds provides a real rapid testing protocol \textit{(see supplementary S8)}.

Multiple samples with slightly different hydrodynamic diameters were prepared and measured in-vitro with the aim of detecting commercially available SARS-CoV-2 specific antibodies. For the \textbf{binding sample (S+)} SARS-CoV-2-S1 protein was covalently bound to the surface of MNPs functionalized with (3-Aminopropyl)tiethoxysilan (APTES) using a protocol modified from \cite{18} and resulting in MNP-APTES-S1. The preparation of the samples for the measurements were the following \textit{(see supplementary S3)}: antibodies were diluted 1:2,000...200,000 (3.3 pM...33 pM) in a buffer (PBS with 0.1% BSA). For each measurement, 25 $\mu$l antibody dilution or reference sample (dilution buffer) were added to 25 $\mu$l of MNP-APTES-S1 dispersions (100 $\mu$g Fe/ml) in an 0.5 ml Eppendorf cap. After adding the antibody dilution or buffer (reference sample), the samples were mixed shortly by pipetting and directly measured without any further incubation time. The \textbf{reference sample (ref)} contained the MNP-APTES-S1 and a buffer solution.

In Fig. 3 a&b, exemplary the real and imaginary parts of the 3rd harmonic of two experiments with two samples each, in dependency of a step-wise increased offset magnetic field $H_{\text{DC}}$ are shown. These ‘full data sets’ were acquired with a benchtop MPS device with adjustable offset magnetic field system. The
amount of single data sets includes 7,200 single measurements per sample and required several minutes acquisition time each (see supplementary S4).

A closer look at the nodes of each experiment revealed differences between the crossing points of the samples. The reference-vs-reference measurement (ref₁ & ref₁', Fig. 3 a) showed almost no difference in the signal demonstrating the stability of the measurement. In contrast, the difference between a reference and a binding-sample (ref₁ & St, Fig. 3 b) while subtle was clearly detectable. In Fig. 3 c, the differences between both experiments are indicated (amplitude differences and phase differences). Two prominent results became evident: first, the difference in the peak height and width of the phase differences (solid line) between both experiments. The phase difference between a reference and binding sample measurement is by a factor $f_{\text{dp}} \approx 17$ increased compared to the phase difference of two reference samples. Second, the height of the amplitude difference (dashed lines), especially in the range of the peak, differed strongly and approaches zero for the ref₁ & ref₁' measurement. Including this calculated amplitude difference factor $f_{\text{dA}}$ of about 10 would also help to distinguish noise from true signal changes in the vicinity of the critical points.

The phase difference for the ref₁ & ref₁' measurement also showed a clearly visible peak, which lay, as expected, at the highest phase sensitivity of the system (critical point). This effect is dominated by noise and slightly by systematic errors such as sample positioning between the successively performed measurements. This reflects an intrinsic sensitivity limit of the used device.

The initial result in Fig. 3 revealed not only a high sensitivity on minimal changes of particle diameters (mobility) in the vicinity of each CP for each higher harmonic but also indicated a high robustness on hardware requirements or magnetic field parameters due to the width of the peak.

**Mobile COMPASS device**

Many measuring techniques are based on physical effects and their sensitivity commonly correlates with the complexity of the underlying measurement hardware. With increasing demand on sensitivity, the requirements for sophisticated hardware to guarantee the necessary specificity and robustness increase significantly. Thus, depending on the desired application, such methods may become non-feasible.

The observed results suggest design parameters and design specifications for a highly flexible and robust device allowing very sensitive and specific measurements. The device presented in the following is based on common MPS technology running at a base-frequency $f_1=20$ kHz and comes with a robust hardware design and easy-to-handle experiments [6]. Based on the results shown above, an important hardware modification was introduced. By adding a strong permanent magnet, which generates a strong magnetic field gradient $G$ along the measurement area providing a range of offset magnetic fields $H_{\text{DC}}$ within the sample volume. Under the right condition between excitation field $H_{\text{AC}}$ and offset magnetic fields covering one or more CPs, the sensitivity of MPS experiments against minimal changes in mobility was improved significantly (see supplementary S2).
In Fig. 4 a, the proposed modified MPS device is shown. As a mobile and highly flexible stand-alone device, it consists of the main control device with all required electronic parts, such as transmit/receive (tx/rx) module for generating the required magnetic fields and measuring the sample signals as well as a battery pack as power supply. The sketch in Fig. 4 b of the tx/rx module shows a cross-section through the tx/rx module indicating the position of the sample in the field of view (FOV) in the center of one of the receive coil pair (rx) wired as gradiometer, the transmit solenoid (tx) and the permanent magnet. The offset magnetic field $H_{\text{DC}}(x)$ generated by the permanent magnet creates a strong magnetic field gradient $G$ within the FOV (see supplementary S5), which influences the inductively measured signal significantly.

In Fig. 5 a, the first results of the proposed mobile modified MPS device measuring the binding state of MNP-APTES-S1 particles are shown (for data processing details see supplementary S6). Each measurement was performed 5 times without averaging. The sequence of samples was reference sample (ref) containing buffer, binding sample (S+) containing a S1 binding antibody (SARS-CoV-2-S1 antibody) and non-binding-sample (S-) containing a non-binding antibody (MERS-CoV-S1 antibody) and was repeated 2 times resulting in 30 individual measurements. The acquisition time for each experiment was 10 ms with a minimum repetition time of 1 s. The graph shows the phase difference $d\varphi_n$ on selected higher harmonic ($n=2^{\text{nd}}$ to $9^{\text{th}}$) against the reference sample. A significant phase difference on each harmonic was observed for the binding sample (S+) but not for the non-binding sample (S-). Here, the $9^{\text{th}}$ harmonic showed the highest difference. But also other harmonics revealed significant phase differences since the applied gradient (permanent magnet) ensured a broad range of offset magnetic fields acquiring signals from multiple critical points.

For comparison, a series of experiments were performed to demonstrate the influence of the magnetic offset fields and magnetic field gradient on the signal (Fig. 5 b). For that, the same experiment sequence was performed for three different cases: (1) with permanent magnet in described position, (2) with permanent magnet in a rotated position (90° degrees against the tx/rx orientation) and (3) without permanent magnet. It became evident, that in case (1) and (2) (with permanent magnets) the desired signal (phase difference) was more prominent than without (case (3)). The signals with permanent magnets differed depending on the gradient strength generated by the permanent magnet within the FOV. This variation depended on the range of offset fields (gradient G) mentioned above.

**Can COMPASS be an alternative to ELISA and flow cytometry?**

The results in Fig. 5 with the modified MPS device represent the signal not only at one specific position $H_{\text{DC}}$ of the Chebyshev-like polynomial (Fig. 3) but the integration of signals over a range of offset magnetic fields (supplementary S2). The sensitivity strongly depends on the chosen gradient field $G$ as indicated in Fig. S2-2. As shown in Fig. 3, the sensitivity of the method for specific harmonics increased further by adjusting the gradient field around a very small and specific range covering the area of a specific critical point $CP_{i,j}$ (see S5-4) but potentially at the cost of reduced robustness.
However, despite the simple setup, the sensitivity of the mobile COMPASS device reaches ~5 ng/ml (0.85 fmole) of SARS-CoV-2-S1 IgG antibody (see Fig. 6), which is comparable to the sensitivity of flow cytometry devices (100-200 ng/ml) as well as the sensitivity of ELISA tests (20-40 ng/ml) [15] (supplementary S7).

More important, the handling of COMPASS experiments and measurements are more flexible and requires no complicated sample preparation and the results are robustly available in shorter protocol time (seconds Vs. hours) including conjugation time. Furthermore, a quantification of the amount of bindings on the surface of the functionalized particles was observed with COMPASS at a high specificity (supplementary S7).

Our method can be used as a robust, fast and easy-to-handle and cheap testing method for sensitive and specific antigen or antibody determination. It thus offers a wide variety of applications in clinical chemistry and biomedical analytics.

COMPASS also allows the measurement of intermolecular interactions of different compartments on functionalized magnetic particles. This opens a wide field in physics, medicine, biology and chemistry [10, 20].

Since the basic effect of the presented COMPASS method is based on a magnetic offset field induced effect, which occurs for excitation magnetic fields $H_{AC}(t)$ as well as offset magnetic fields $H_{DC}$ with magnitudes of at least 2-3 mT or above, this technique differs from common MPS and ACS experiments (supplementary S4). Setting up a critical point by adjusting the AC and DC magnetic fields enables the measurement of minimal changes in the effective mobility of the samples. The high sensitivity at the critical point is caused by a kind of background suppression, where background can be defined as signal from particles unaltered in their mobility in the presence of the analyte.

In addition, the differential measurement (sample Vs reference) of the phases can overcome issues in signal interpretation occurring in MPS or ACS experiments due to concentration dependencies. This provides a huge list of particle parameters accessible with high accuracy, which can be seen in the Langevin equation (EquS. 2-1) consisting of multiple parameters such as the magnetic moment of the particle $m$, the Temperature $T$ and the friction $\zeta$, where the latter is the product of viscosity $\eta$ of the surrounding medium, the hydrodynamic particle radius $R_H$ and the particle shape $\kappa$. The advantage of this method is the direct access to particle parameters, which are of high interest for understanding the complex dynamics of MNP ensembles. Furthermore, fast and easy access to these parameters allows a robust MNP characterization during synthetization and hence gives immediate feedback of improving the quality of magnetic particles, e.g., for medical applications or environmental treatment [21, 22].

Beyond the aforementioned, many more applications in different fields of research are conceivable, and COMPASS will pave the way for their realization.

Declarations
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Authors Contributions

P.V.: initial idea, hardware development, assembling spectroscope, performing experiments, preparing figures, data processing, provides software for data processing and simulations, writing the manuscript. M.A.R.: initial idea, hardware developing, assembling spectroscope, evaluating theory, writing the manuscript B.F.: sample preparation, experimental design, performing experiments, data processing, writing the manuscript R.T.: initial idea, sample preparation, experimental design, writing the manuscript S.L.: initial idea, sample preparation, experimental design, writing the manuscript T.K.: evaluating theory, writing the manuscript T.H.: experimental design L.D.: initial idea, experimental design, writing the manuscript C.A.: providing chemistry and biology labs, resource management V.C.B.: providing MPI lab, resource management, writing the manuscript. All authors reviewed the manuscript.

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Figures

Figure 1

ACS and common MPS are using either weak AC and strong DC magnetic fields (static or with low frequency \( f_{AC} \)) or strong AC and weak DC magnetic fields. At critical DC magnetic field offsets in the strong AC & DC magnetic field regime \( (H_{DC}<H_{AC}) \), the signal phase can be especially sensitive to small changes in the MNP mobility – Critical Offset Magnetic PArticle SpectroScopy (COMPASS).

Figure 2

(a)&(b): The behavior of the magnetization \( M \) of MNPs in dependency of external magnetic fields \( H \) can be described by the non-linear Langevin function (red). Exposing an MNP ensemble to a sinusoidal magnetic field \( H_{AC}(t) \) with frequency \( f_1 \) and sufficient amplitude, the magnetization response \( M(t) \) consists not only of the fundamental frequency but also odd (and even) higher harmonics \( (f_n=n\cdot f_1) \) depending on the presence of an offset magnetic field \( H_{DC} \), which can be visualized in the Fourier spectrum.

(c) Visualizing the dependency of the harmonic \( A_n \) for the \( n \)-th higher harmonic for increasing offset magnetic field \( H_{DC} \) (with \( H_{DC}<H_{AC} \)). The specific shape for varying \( H_{DC} \) with nodes (green arrow) depends on the harmonic number \( n \). As an example, the real part of the 3\(^{rd} \) harmonic of simulated data is indicated to show the connection between a ‘dip’ in the Fourier spectrum and a ‘node’ in the \( A_n(H_{DC}) \) plot: this point is called critical point (CP).

(d) In the vicinity of a CP, the position which is most susceptible to the sample parameters, the phase \( \varphi(H_{DC}) \) of the signal shows an approx. 180\(^{o} \) degree shift with a strong slope. Thus, even minimal changes in the sample parameters and thus in the shape of the \( A_n(H_{DC}) \ & \varphi(H_{DC}) \) curves result in high changes in the phase \( \varphi(H_{DC}) \) and thus in the resulting signal. The 6\(^{th} \) and 9\(^{th} \) harmonic are integer multiples of three and therefore happen to also vanish in this case (grey arrows).

Figure 3
(a)&(b): the real and imaginary part for the 3rd harmonic ($H_{AC}=17$ mT) of experiments with a reference sample ($\text{ref}_1$ & \text{ref}_1'$) and a reference sample and a binding sample ($\text{ref}_1$ & $S+$). The differences in the crossing points of real and imaginary data are clearly visible. (c) the phase difference (solid lines) as well as the amplitude difference (dashed lines) of both experiments differentiate binding sample ($\text{ref}_1$ & $S+$) from control ($\text{ref}_1$ & $\text{ref}_1'$).

**Figure 4**

(a) mobile COMPASS device with the main device consisting of all necessary hardware components such as microcontroller, amplifier and filters, the transmit/receive module for 0.5 ml Eppicaps. (b) A closer look at the coil design within the tx/rx module indicates the positioning of the samples within one of the gradiometric receive coils. (c) The offset magnetic field $H_{DC}$ along the FOV shows a strong gradient $G$, which has massive influence on the signal differentiation of binding and non-binding states of functionalized MNPs (d).

**Figure 5**

(a) First measurements of mobile modified MPS device on binding states on MNP-APTES-S1. The measurement sequence was 5 times reference sample (ref), 5 times binding sample ($S+$) and 5 times non-binding sample ($S-$) and was repeated two times resulting in 30 individual experiments. The phase difference for selected higher harmonics are clearly visible.

(b) Comparison of signals with and without offset magnetic fields (gradient field). All sequences show 5 experiments with reference sample (ref), 5 with binding sample ($S+$) and 5 with non-binding sample ($S-$) repeated two times. Left: signal with a permanent magnet, center: magnet rotated. Right: signal without any external offset or gradient field.

**Figure 6**

Results of the measurements with an optimized setup (see supplementary S5) on the 5th harmonic of the binding states of a dilution of 1:200,000 SARS-CoV S antibodies (33 pM) on MNP-APTES-S1. The measurement sequence was 5 times reference sample (ref) and 5 times binding sample ($S+$) and was repeated three times resulting in 30 individual experiments. The acquisition time was 10 ms per
experiment, the prior preparation time including mixing and incubation of samples and particles was below 1 minute.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- COMPASSsupplementary.docx
- anim3rd.gif
- anim7th.gif