**Plasma pS129-⚜-synuclein is a surrogate biofluid marker of motor severity and progression in Parkinson's disease**

**SUPPLEMENTARY INFORMATION**

**Measurement of plasma pS129-⚜-synuclein**

To assay plasma pS129-◎-synuclein, we established the reagents for measuring pS129-◎-synuclein in plasma: dextran-coating magnetic $Fe_3O_4$ nanoparticles (MF-DEX-0060, MagQu) bio-functionalized with monoclonal antibodies (825701, Biolegend) against phosphorylated Ser129 ◎-synuclein.[1, 2] The mean diameter of the antibody-immobilizing magnetic nanoparticle was 56 nm, as detected by laser dynamic scattering (SZ100-S, HORIBA). The concentration of pS129-◎-synuclein reagent was 8 mg Fe/ml, with 60 µl of reagent mixed with 60 µl of plasma for measurement of the pS129-◎-synuclein concentration by IMR.

The reagent for IMR consists of magnetic nanoparticles functionalized with antibodies against pS129-◎-synuclein dispersed in phosphate-buffered saline (pH 7.2). The reagent was superparamagnetic with the saturated magnetization of 0.3 emu/g. After mixing the reagent and the tested plasma sample, each mixture was placed into a superconducting quantum interference device (SQUID)-based alternative current (ac) magnetosusceptometer (XacPro-S, MagQu) to determine the time-dependent ac magnetic susceptibility that approximates the association between magnetic nanoparticles and pS129-◎-synuclein protein molecules in plasma. Because of the association between the antibody-functionalized magnetic nanoparticles and target biomarkers, the ac magnetic susceptibility of the mixture was reduced. This reduction in the magnetic susceptibility due to the association between a magnetic nanoparticle and pS129-◎-synuclein molecule can be sensed by a high-Tc SQUID magnetometer and is referred to as the IMR signal. The dynamic range of the plasma level of pS129-◎-synuclein was measured using the changes in IMR signals, which were denoted as $\varphi_{\alpha\text{-syn,IMR}}$. Therefore, the IMR signal is a function of the concentration of pS129-◎-synuclein. Duplicate measurements were performed for IMR signals at each concentration of plasma pS129-◎-synuclein.

Standard samples of varying concentrations of pS129-◎-synuclein⚜ peptides (ab188826, Abcam) in phosphate-buffered saline (PBS) were used to detect pS129-◎-synuclein concentration-dependent IMR signals before testing plasma samples from participants. The concentration of pS129-◎-synuclein solution ranged from 0.483 fg/ml to 144.8 pg/ml (Supplementary Table 1). The IMR signal for PBS only was measured as a blank control. The IMR signals for varying concentrations of pS129-◎-synuclein solutions obtained from triplicate measurements (i.e., IMR(%)-$\phi_{pS129}$) are tabulated in Supplementary Table 1. The data points in Supplementary Figure 1A follow the logistic function in Eq. 1:

$$\text{IMR}(\%)=\frac{\text{A-B}}{1+\left(\frac{\varphi_{pS129}}{\varphi_o}\right)^{\gamma}}+\text{B}, \qquad \text{(Equation 1)}$$

where A, B, $\phi_o$, and $\gamma$ are fitting parameters and $\phi_{pS129}$ is the concentration of standard pS129-◎-synuclein peptide solution. By fitting the individual known standard pS129-◎-synuclein peptide concentration data to Equation 1 and the measured IMR signals, the parameters were 2.637, 4.138, 0.635, and 0.602 for A, B, $\phi_o$, and $\gamma$, respectively. Specifically, A denotes the IMR signal of blank control, B denotes the saturated IMR signal as $\phi_{pS129}$ approaches infinity, and $\phi_o$ is the pS129-◎-synuclein peptide concentration. Using this standard curve, the Hook effect, low detection limit, and assay range of plasma pS129-◎-synuclein concentrations using IMR can be determined as described in below.

**Measurement of pS129-⚜-synuclein concentration by IMR signals**

**Hook effect**

The IMR signal of the standard pS129-⊚-synuclein peptide concentration at 144,780 fg/ml (Supplementary Table 1) is below the fitting curve (Supplementary Figure 1A, right most data point). The deviation of data points from the fitting curve at this highest concentration contribute to the Hook effect. Thus, the Hook effect occurs when the pS129-⊚-synuclein peptide concentration is >48,260 fg/ml.

Detection limitation of the IMR assay for plasma pS129-⊚-synuclein

The global standards for the assay detection limit are described in Clinical & Laboratory Standards Institute (CLSI) Guidelines EP17-A2. According to CLSI EP17-A2, the limit of blank (LoB) should be established first, then the limit of detection (LoD). The LoB is the value at the appropriate percentile (p) of all ranked measured values (Eq. 2).

LoB = Results at position [p × NB + 0.5] (Equation 2)

where NB is the number of trials. In the current study, NB = 60 and p = 0.95 (Eq. 3).

LoB = Results at position 57.5 (Equation 3)

This is a non-integer value. The distribution of 60 testing results is non-Gaussian. Linear interpolation is carried out using the 57th and 58th ranked observations according to CLSI EP17-A2. The 60 measured concentrations for PBS samples are blank controls and ranked in Supplementary Table 2. Using the 57th and 58th ranked values for the linear interpolation, the 57.5th observation indicates that the measured concentration is $2.89\times10^{-4}$ fg/ml.

The LoD is calculated according to Eq. 4:

$LoD = LoB + 1.645\ \sigma_S$ (Equation 4)

where $\sigma_S$ is the standard deviation of the measured pS129-⊚-synuclein concentration at a given spiked pS129-⊚-synuclein peptide concentration (0.5 fg/ml in this study). The pS129-⊚-synuclein concentrations of 60 tests for this known concentration of pS129-⊚-synuclein peptide sample are listed in Supplementary Table 3. The mean of 60 repeated measurements was 0.542 fg/ml. The $\sigma_S$ of the 60 measured concentrations was $4.39\times10^{-2}$ fg/ml. The LoD for assaying pS129 was $7.24\times10^{-2}$ fg/ml using Equation 4.

**IMR assay range for plasma pS129-✻-synuclein**

The detected IMR signals are converted to measured pS129-⊚-synuclein concentration, denoted as $\phi_{pS129\text{-}m,}$ using Equation 1 (Supplementary Table 1). As the sample with a concentration of 144.8 pg/ml demonstrates a Hook effect, its IMR signal is not counted. The relationship between the measured pS129-⊚-synuclein concentration and spiked pS129 concentration is shown in Supplementary Figure 1B. A proportionality was found (solid line in Supplementary Figure 1B) with a slope of 0.9575. The coefficient of determination ($R^2$) was 0.999. According to CLSI guideline EP06-A2, the valid range for the slope is 0.9 to 1.1. The slope for the proportionality in our assay is within the valid range. Therefore, the measurement range for detecting plasma pS129-⊚-synuclein using IMR is 0.483 fg/ml to 46.3 pg/ml.

**Interference tests**

One plasma sample from a PD patient was assayed using IMR. The measured pS129-⊚-synuclein, denoted as $\phi_{pS129\text{-}m}$, was 54.9 fg/ml. This concentration was used as a reference to investigate the interference effect of abundant protein molecules in human plasma, such as bilirubin, hemoglobin, intralipid, albumin, rheumatoid factor, uric acid, and α-synuclein, on the assay of pS129-⊚-synuclein. The concentrations of these protein molecules are listed in Supplementary Table 4. The recovery rate, defined as the ratio of the measured pS129-⊚-synuclein concentration of the sample mixed with individual common blood protein molecules compared to the original sample, is given in Supplementary Table 4. The recovery rate for each condition ranged between 90% and 100%, revealing no significant interference in detecting the plasma pS129-⊚-synuclein concentration by these common protein molecules in venous blood.

**Supplementary Table 1.** Measured IMR signals for variable concentrations of standard pS129-⊙-synuclein peptides in PBS solution.

| Standard pS129-⚜-synuclein peptide concentration, fg/ml | Measured IMR signals, % | Converted pS129-⚜-synuclein concentrations from IMR signals ($\phi_{sP129\text{-}m}$), fg/ml |
|---|---|---|
| 0 | 2.532 ± 0.029 | - |
| 0.4826 | 2.656 ± 0.0001 | 0.4524 |
| 4.826 | 2.710 ± 0.0017 | 4.586 |
| 48.26 | 2.906 ± 0.0008 | 50.81 |
| 482.6 | 3.318 ± 0.0018 | 469.2 |
| 4826 | 3.801 ± 0.0054 | 5021 |
| 48,260 | 4.032 ± 0.0008 | 46,170 |
| 144,780 | 4.052 ± 0.0003 | - |

**Supplementary Table 2.** Ranking of the 60 test results for pS129-◎-synuclein concentrations in PBS blank controls using the IMR assay.

| Rank | Measured concentration, fg/ml | Rank | Measured concentration, fg/ml |
|---|---|---|---|
| 1 | -0.763429 | 31 | -0.044445 |
| 2 | -0.717388 | 32 | -0.018046 |
| 3 | -0.672437 | 33 | -0.018046 |
| 4 | -0.585886 | 34 | -0.018046 |
| 5 | -0.585886 | 35 | -0.018046 |
| 6 | -0.544329 | 36 | -0.018046 |
| 7 | -0.464764 | 37 | -0.018046 |
| 8 | -0.426806 | 38 | -0.018046 |
| 9 | -0.426806 | 39 | -0.018046 |
| 10 | -0.426806 | 40 | -0.018046 |
| 11 | -0.390101 | 41 | -0.008580 |
| 12 | -0.390101 | 42 | -0.008580 |
| 13 | -0.390101 | 43 | -0.008580 |
| 14 | -0.390101 | 44 | -0.008580 |
| 15 | -0.320568 | 45 | -0.008580 |
| 16 | -0.320568 | 46 | -0.008580 |
| 17 | -0.320568 | 47 | -0.008580 |
| 18 | -0.287804 | 48 | -0.008580 |
| 19 | -0.256422 | 49 | -0.002153 |
| 20 | -0.197970 | 50 | -0.002153 |
| 21 | -0.170990 | 51 | -0.002153 |
| 22 | -0.170990 | 52 | -0.002153 |
| 23 | -0.145576 | 53 | -0.002153 |
| 24 | -0.145576 | 54 | -0.002153 |
| 25 | -0.121789 | 55 | -0.002153 |
| 26 | -0.099695 | 56 | 0.000289 |
| 27 | -0.079375 | 57 | 0.000289 |
| 28 | -0.079375 | 58 | 0.000289 |
| 29 | -0.060920 | 59 | 0.000289 |
| 30 | -0.060920 | 60 | 0.000289 |

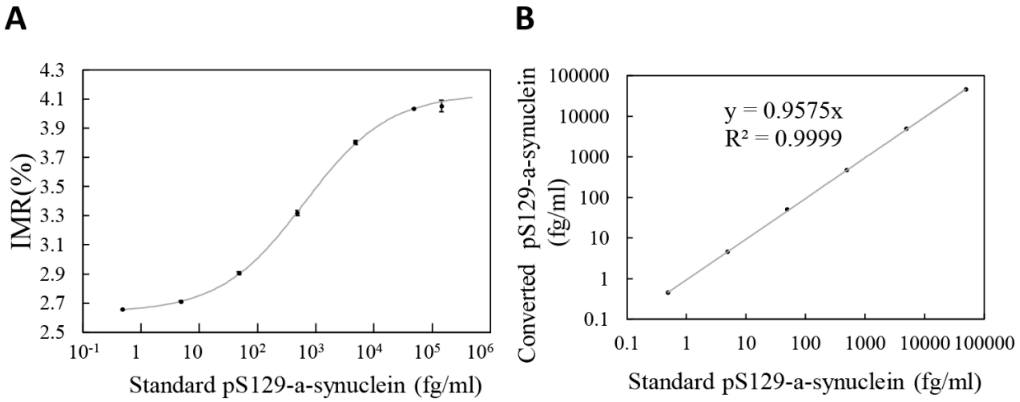
**Supplementary Table 3.** Ranking of the 60 test results for pS129-◎-synuclein concentrations in a known sample of pS129-◎-synuclein peptide solution (0.5 fg/ml) using the IMR assay.

| Rank | Measured concentration, fg/ml | Rank | Measured concentration, fg/ml |
|---|---|---|---|
| 1 | 0.467236 | 31 | 0.537578 |
| 2 | 0.467507 | 32 | 0.53886 |
| 3 | 0.475874 | 33 | 0.54753 |
| 4 | 0.479097 | 34 | 0.547530 |
| 5 | 0.479224 | 35 | 0.547530 |
| 6 | 0.479697 | 36 | 0.549316 |
| 7 | 0.482177 | 37 | 0.549493 |
| 8 | 0.491903 | 38 | 0.550107 |
| 9 | 0.492844 | 39 | 0.551834 |
| 10 | 0.493673 | 40 | 0.555208 |
| 11 | 0.499215 | 41 | 0.574200 |
| 12 | 0.501259 | 42 | 0.579725 |
| 13 | 0.502749 | 43 | 0.583443 |
| 14 | 0.503159 | 44 | 0.585789 |
| 15 | 0.504759 | 45 | 0.585789 |
| 16 | 0.504759 | 46 | 0.587215 |
| 17 | 0.508770 | 47 | 0.588221 |
| 18 | 0.508834 | 48 | 0.588255 |
| 19 | 0.513938 | 49 | 0.590625 |
| 20 | 0.519092 | 50 | 0.591708 |
| 21 | 0.520618 | 51 | 0.591736 |
| 22 | 0.521053 | 52 | 0.594736 |
| 23 | 0.521053 | 53 | 0.595085 |
| 24 | 0.521381 | 54 | 0.598038 |
| 25 | 0.522615 | 55 | 0.600296 |
| 26 | 0.524816 | 56 | 0.603638 |
| 27 | 0.526960 | 57 | 0.613141 |
| 28 | 0.529032 | 58 | 0.614592 |
| 29 | 0.533091 | 59 | 0.615892 |
| 30 | 0.535211 | 60 | 0.617305 |

**Supplementary Table 4.** Interference tests for IMR assay of plasma pS129-⍟-synuclein.

| Sample No. | Contents | Measured pS129, fg/ml | Recovery rate, % |
|---|---|---|---|
| 1 | Human plasma | 54.9 | - |
| 2 | Human plasma with 10,000 μg/ml bilirubin | 50.3 | 91.6 |
| 3 | Human plasma with 30,000 μg/ml hemoglobin | 49.9 | 90.9 |
| 4 | Human plasma with 60,000 μg/ml intralipid | 52.6 | 95.8 |
| 5 | Human plasma with 500 IU/ml albumin | 53.3 | 97.1 |
| 6 | Human plasma with 200 μg/ml rheumatoid factor | 56.7 | 103.3 |
| 7 | Human plasma with 10 pg/ml uric acid | 53.1 | 96.7 |
| 8 | Human plasma with 10 pg/ml α-synuclein | 57.8 | 105.3 |

**Supplementary Figure 1.** The relationship between the IMR signal (A) or the measured pS129-◎-synuclein concentration (B) and the standard pS129-◎-synuclein peptide concentration.



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