Supporting Information

Biosensing Tacrolimus in Human Whole Blood by Using a Drug Receptor Fused to the Emerald Green Fluorescent Protein

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Figure S1. Chemical structures of the immunosuppressant drugs tacrolimus (FK506), mycophenolic acid (MPA) and sirolimus (Sir), and the carboxylated derivative of tacrolimus (FK506-CO$_2$H).

Table S1. Primer sets used for PCR amplification of the pQE-T7-2 vector and gene fragments. The oligo tail added to the target gene sequence is in bold face, the segment identical with the target gene in italics and the overlapping regions for the Gibson assembly reaction underlined.

| Vector          | Primer | Sequence 5′-3′          |
|-----------------|--------|-------------------------|
| FKBP1A plasmid  | FP     | ATC ACC ATG AAA ACC TGT ATT TTC AGT CC |
|                 | RP     | TTG CTC ACC GAA CCT CCA CCT |
| EmGFP plasmid   | FP     | GGT GGA GGT TCG GTG AGC AA |
|                 | RP     | GCA GCC GGA TCT TAC TG |
| pQE-T7-2 backbone| FP     | TAG TAA GAT CCG GCT G |
|                 | RP     | ATA CAG GTT TTC ATG GTG ATG CAT ATG ATT |

Figure S2. Alignment of the gene sequence of the recombinant FKBP-EmGFP protein (bottom scheme) with the sequences obtained by the Sanger sequencing with the direct (blue line) and reverse (red line) primers. The colored parts of the upper arrows indicate the homologous areas of the sequences. Sanger sequencing was carried out at the Genomics and Proteomics Research Support Center of Complutense University and the alignment was done with the SnapGene 3.2.1 software (GSL Biotech).
Figure S3. SDS-PAGE analysis of the purified fusion protein with Coomassie brilliant blue protein staining: lane 1 and 9, molecular marker (Thermo Scientific™ PageRuler™ Prestained Protein Ladder, 10 to 180 kDa); 2, lysate sample; 3, HisTrap column throughput with non-retained proteins; 4-7, aliquots from the HisTrap column elution; 8, purified protein obtained after PD-10 column. Prior to analysis, a 5-minute boiling step at +95 °C have been used. The thickest band corresponds to the molecular weight of the fusion protein (~40.6 kDa).

Figure S4. Fluorescence decay profile of the FKBP1A-EmGFP protein in PBS at 25 °C. IRF: Instrument Response Function.
Protein identification by MALDI-TOF mass spectrometry

MALDI-TOF MS analysis of the electrophoretically resolved protein was performed, at the Proteomics Unit of the Complutensian University of Madrid, on a 4800 Plus Proteomics Analyzer MALDI-TOF/TOF mass spectrometer (Applied Biosystems, MDS Sciex, Toronto, Canada). The protein was excised from the SDS-PAGE gel, in-gel reduced, alkylated and digested with trypsin. The MALDI-TOF/TOF MS technique provides peptide mass fingerprints (Figure S5A), those peptides were collated and represented as a list of monoisotopic molecular weights and compared with the masses of theoretical trypsin digestion of the recombinant protein (Figure S5B and Table S2). The results showed a coverage of 63% of the protein, having the FKBP1A domain a coverage of more than 87% of the primary sequence. Furthermore, the EmGFP domain was also observed except the C-terminal end that contains low number of Lys and Arg avoiding the digestion by Trypsin that produces too large peptides to be identified by MS.

Table S2. Information of peptides identified form the recombinant FKBP1A-EmGFP after a trypsin digestion and MALDI-TOF MS analysis.

| Start - End | Observed | Mr(expt) | Mr(calc) | ppm  | M'   | Peptide                                              |
|-------------|----------|----------|----------|------|------|------------------------------------------------------|
| 1-27        | 3149.4546| 3148.4473| 3148.4435| 1.23 | 0    | .-MHHHHHENLYFQSGVQVETISPGDGR.T                    |
| 1-27        | 3165.4644| 3164.4571| 3164.4384| 5.91 | 0    | .-MHHHHHENLYFQSGVQVETISPGDGR.T + Oxidation (M)    |
| 32-48       | 1950.9281| 1949.9208| 1949.9088| 6.18 | 1    | K.RGQTCVHYTGMLEDGK.K                                |
| 32-48       | 1966.9147| 1965.9074| 1965.9037| 1.88 | 1    | K.RGQTCVHYTGMLEDGK.K + Oxidation (M)                |
| 33-48       | 1794.8073| 1793.8000| 1793.8077| -4.29| 0    | R.GTCVHYTGMLEDGK.K                                 |
| 33-49       | 1922.8994| 1921.8921| 1921.9026| -5.46| 1    | R.GTCVHYTGMLEDGK.K                                 |
| 55-61       | 904.4854 | 903.4781 | 903.4926 | -16.1| 1    | R.DRNKPFK.F                                        |
| 62-71       | 1220.6844| 1219.6772| 1219.6747| 2.03 | 1    | K.FMLGKQEVR.G                                      |
| 67-85       | 2159.0652| 2158.0579| 2158.0589| -0.47| 1    | K.KEVIRGWEVGQAQMSVGQR.A                            |
| 72-85       | 1533.7186| 1532.7114| 1532.7042| 4.68 | 0    | R.GWEEGVAQMSVGQR.A                                 |
| 72-85       | 1549.7126| 1548.7054| 1548.7077| -4.29| 0    | R.GTCVHYTGMLEDGK.K                                 |
| 88-119      | 3405.7754| 3404.7681| 3404.8020| -9.96| 0    | K.LTISPDYAYGATGHPGIIPPHATLVFDVELLL.K                |
| 129-151     | 2437.2913| 2436.2840| 2436.2537| 12.4 | 1    | K.GEELFTGVVPLVEDGVDVNHK.F                          |
| 152-166     | 1503.6492| 1502.6419| 1502.6525| -7.08| 0    | K.FSVSGEVEGDTAYKG.L                                |
| 152-170     | 1598.9813| 1597.9740| 1597.9633| 5.50 | 1    | K.FSVSGEVEGDTAYGKLTLK.F                            |
| 199-210     | 1608.7136| 1607.7063| 1607.7191| -7.93| 1    | R.YPDHMKOHDFK.S + Oxidation (M)                     |
| 211-221     | 1266.5757| 1265.5684| 1265.5710| -2.06| 0    | K.SAMPEGYVQR.T                                     |
| 211-221     | 1282.5677| 1281.5605| 1281.5659| -4.26| 0    | K.SAMPEGYVQR.T + Oxidation (M)                      |
| 222-232     | 1347.6522| 1346.6449| 1346.6507| -4.25| 1    | R.TIFFKDDGYK.T                                     |
| 235-247     | 1477.7542| 1476.7469| 1476.7572| -7.02| 1    | R.AEVKFEDTLVNR.I                                   |
| 239-247     | 1050.5089| 1049.5016| 1049.5142| -11.9 | 0    | K.FEGDLVNR.I                                       |
| 252-265     | 1542.7926| 1541.7853| 1541.7838| 0.99 | 1    | K.GIDFKEDGNILGHK.L                                 |
Table S2 (continued).

| 257-274 | 2130.9939 | 2129.9866 | 2130.0130 | -12.4 | 1 | K.EDGNILGHKLEYNYNSHK.V |
| 266-274 | 1167.5317 | 1166.5245 | 1166.5356 | -9.55 | 0 | K.LEYNYNSHK.V |
| 275-283 | 1065.5996 | 1064.5923 | 1064.5866 | 5.39  | 1 | K.VYITADKQK.N |

*M*: missed cleavage sites.

Figure S5. (A) MALDI-TOF mass spectrum of the FKBP1A-EmGFP eluted protein. (B) Sequence coverage of matched peptides (bold red) in FKBP1A-EmGFP. Marked amino acids correspond to the theoretical cleavage sites of trypsin enzyme (C-term side of K and R).
Preparation of the FK506-protein platform on the QCM sensor and data analysis

QCM data analysis. The sensed mass of adsorbed protein layers was estimated using the Sauerbrey equation (Eq. S1) and the Voigt model supplied by QCMBrowser 2.30 software (KSV Instrument Ltd, Helsinki, Finland).

\[ \Delta f = -\frac{2nf_0^2}{A\sqrt{\rho_q\mu_q}} \Delta m \]  
\[ \text{Eq. S1} \]

where \( n \) is the overtone number and \( \rho_q \) and \( \mu_q \) are the density and the shear modulus of the quartz crystal, respectively.

While the Sauerbrey equation was used for calculating the sensed mass of the rigid film with small or no energy dissipation, the Voigt model was applied in the case of larger dissipation values corresponding to soft and elastic layers.

The surface density of the sample at saturation, and the relative molecular area occupied on the surface, \( \bar{A} \), was obtained from Eq. S2

\[ \bar{A} = \frac{MW}{(\Delta m/A)_{Sat}} N_A \]  
\[ \text{Eq. S2} \]

where \( MW \) is the molecular weight of the adsorbed molecule, \( (\Delta m/A)_{Sat} \) is the surface mass density at monolayer saturation and \( N_A \) is the Avogadro number.

Thickness of the adsorbed layer was determined from the detailed analysis of all overtones for the QCM-Z set-up using the QCMBrowse software and by simple estimation of the layer thickness using an average density of the adsorbed proteins obtained from QCMBrowse data fitting.

The data were analyzed with Igor 6.10A software (Wavemetrics, Lake Oswego, OR, USA).

The calculation of the kinetic parameters was done with a Langmuir binding model (Eq. S3):

\[ \frac{\Delta m}{A} = \frac{(\Delta m/A)_{Sat}[\text{FK506}]}{k'_d+[\text{FK506}]} \]  
\[ \text{Eq. S3} \]

where \( (\Delta m/A)_{Sat} \) is the saturation surface mass density, \( k'_d \) is the pseudo binding constant for FK506 binding to FKP1A containing proteins.

Formation of dithiobis(C\(_2\)NTA-Ni\(^{2+}\)) nanoplatfroms. Self Assembled Monolayer (SAM) of dithiobis(C\(_2\)NTA)-Ni\(^{2+}\) complex was formed on the QCM sensors using a procedure previously described. Briefly, Au coated AT-cut 5 MHz sensor (Nordtest.srl, Serravalle Scrivia (AL), Italy) were cleaned with a drop of chromic acid solution for a few seconds, rinsed copiously with water and dried with a nitrogen flux. Then, the SAM was obtained with a two-step procedure: the chemisorption of dithiobis(C\(_2\)NTA) covalently immobilized on the sensor surface via sulfur-gold binding, followed by the addition of a Ni\(_2\)SO\(_4\) solution resulting in complexation of Ni\(^{2+}\) to the water-exposed NTA groups.
The average orientation of the dithiobis(C$_2$NTA-Ni$^{2+}$) covalently immobilized on the sensor surface is reported in Figure S6. The procedure provides densely packed and rigid SAM of dithiobis(C$_2$NTA-Ni$^{2+}$) layers on gold-coated QCM sensors, in such nanoplatform Ni-NTA functional groups protrude exposing the two chelating aquoions available for further complexation.

The principal parameters obtained from the adsorption (summarized in Table S3) are in agreement with previous results on the same system which reported a surface mass density of about 95 ng/cm$^2$ and a rigid film thickness of about 16 Å.$^{4,5}$

**Immobilization of FKBP1A-containing protein.** Solution of the fusion proteins (500 nM) in phosphate buffer were added to the QCM measuring chamber containing the dithiobis(C$_2$NTA-Ni$^{2+}$) modified QCM sensor. Consecutive additions were performed until a constant resonance frequency was reached; the chamber was then rinsed with phosphate buffer to remove protein excess or loosely bound protein.

Figure 3B represents the trend of the resonant frequency shift and the variation in energy dissipation of the third harmonic. The decrease in the frequency shift correlates with an increase of adsorbed mass on the sensor indicating that the protein adsorbs on dithiobis(C$_2$NTA-Ni$^{2+}$) layer likely interacting via histidine-tag complexation with the free coordination sites of the surface-immobilized Ni$^{2+}$ ions.$^{6}$ The slight increase of ΔD$_3$ (0.5·10$^{-6}$) shows that adsorption of the protein does not significantly affect the viscoelastic properties of the SAM layer. This finding suggests that a compact protein layer is formed on the surface.

As shown in Figure 3C, the adsorption of the FKBP1A-EmGFP takes around 2.5 h to be completed but the kinetics does not follow a simple mono-exponential dependence on time as expected for Langmuir adsorption. The presence of different kinetic regimes for protein adsorption was observed also by other authors.$^{7}$ In the first regime, the protein is arranged in a flat conformation occupying a large surface area, as adsorption proceeds the flip-up from the flat-lying conformation to a tilted one is promoted, further His-tagged proteins bind to the platform saturating the binding sites. However, the application of this model to our systems was not completely satisfactory, and the best agreement was found using the tri-exponential function (Eq. S4)

$$
\Delta m = y_0 + A_1 e^{\frac{x-x_0}{\tau_1}} + A_2 e^{\frac{x-x_0}{\tau_2}} + A_3 e^{\frac{x-x_0}{\tau_3}}
$$

Eq. S4

where $y_0$ is the saturation value, $x_0$ is the x offset, $A_1$, $A_2$ and $A_3$ are the amplitude and $\tau_1$, $\tau_2$ and $\tau_3$ are the time constants expressed in seconds.

The reported data show that the first two-steps for protein adsorption usually proposed in the literature, namely chemisorption in flat-lying configuration ($\tau_1 = 13.569$ s) and tilting up ($\tau_2 = 168.71$ s) of the molecule for dense monolayer packing, is followed by a slower reorganization of the two protein subunits in the surface layer ($\tau_3 = 1539$ s) to reach the maximum packing density.
The adsorbed mass of the protein, the area occupied by each molecule at surface saturation, the thickness increment caused by the adsorption of the protein and the change in dissipation obtained from the experimental data are reported in Table S3.

The average molecular area for the protein is in accordance with theoretical calculations reported in literature for similar systems containing EmGFP histidine tagged protein, where the average area ranges between 500 and 1250 Å²/molecule depending on the orientation of the protein on the surface.

Further insight in the protein conformation at the interface was obtained from the comparative analysis of the structural experimental data (thickness and molecular area at saturation) with the physical dimensions estimated from the chemical structure of the proteins. Given that the kinetic measurements proved that the proteins do not lay flat at the interface at maximum coverage, such conformations were excluded in the computations. Under this assumption, comparison of the experimental value of Table S3 and the theoretical calculation allows us to draw some general conclusions. Figure S6C reports the most likely conformation of the system, compatible with the multistep kinetic. The FKBP1A protein is vertically oriented while the long axis of EmGFP is oriented with a tilt angle of 10° with respect to the normal to the interface.

**Figure S6.** (A) Chemical structure of the dithiobis(C2NTA-Ni²⁺); (B) schematic representation of the surface density of dithiobis(C2NTA-Ni²⁺) on the Au-QCM sensor, (C) typical representation of the fusion protein conformation at the interface. The 3D structure of dithiobis(C2NTA-Ni²⁺) was obtained by means of the free software Chemsketch (ACD/Labs).

|                          | Dithiobis(C2NTA-Ni²⁺) | HisTag-FKBP1A-EmGFP |
|--------------------------|-----------------------|---------------------|
| Surface mass density (ng/cm²) | 139 ± 20              | 677                 |
| Adsorbed mass (ng)       | 109 ±16               | 531                 |
| Average molecular area (Å²) | 90± 22                | 997                 |
| ΔD₃/3·10⁻⁶              | 0.2 ± 0.3             | 1.0                 |
| Thickness increment, Å   | 16 ± 1                | 66                  |
| Ratio                    | 1.15 ± 0.31           | 10                  |

Table S3. Principal parameters obtained from the adsorption of dithiobis(C2NTA) for the studied system and the FKBP1A modified protein on the C2NTA-Ni²⁺ substrate.
**Binding of FK506 to the FKBP1A-containing protein.** Sequential aliquots of tacrolimus solution at different concentrations ranging between $1 \cdot 10^{-12}$ M and $1 \cdot 10^{-8}$ M in phosphate buffer (10 mM, pH 7.4) were added to the QCM measuring chamber using the protein-functionalized QCM sensor (Figure 3A). $\Delta f$ and $\Delta D$ were recorded as a function of time until constant values were reached for all the examined concentrations. The adsorbed mass after buffer rinsing was obtained from the normalized third overtone of the frequency shift ($\Delta f_3/3$) applying Sauberbrey equation (Eq. S1).
Figure S7. (A) Dilution curve for the FKBP1A-EmGFP as a function of the amount of MB-FK506 (2 – 8 µg/well). (B) Dilution curve for MB-FK506 as a function of the amount of FKBP1A-EmGFP (5.25 µg/mL) in the absence (black symbols, n = 3) and in the presence (opened symbols, n = 3) of 50 µg/mL of FK506. Results are shown as mean signals ± the standard error of the mean (n = 3).

Figure S8. Effect of the buffer composition on the B<sub>50</sub>/B<sub>0</sub> ratio (open symbols). The yellow bars correspond to the fluorescence signal in the absence of FK506 (B<sub>0</sub>), the grey bars correspond to the signal in the presence of 50 ng/mL FK506 (B<sub>50</sub>), and the orange bars to the background signal of the MB-FK506. PBST: phosphate buffer (10 mM, pH 7.4) with T20 (0.05%); PBS: phosphate buffer (10 mM, pH 7.4); HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (10 mM, pH 7.5); TRIS: 2-amino-2-(hydroxymethyl)propane-1,3-diol (10 mM, pH 8.0); MES: 2-(N-morpholino)ethanesulfonic acid buffer (10 mM, pH 4.7); Casein: Blocker™ Casein (1%, w/v) in PBS; PFBB: Pierce protein free (PBS) blocking buffer. Results are shown as mean signals ± the standard error of the mean (n = 3).
Figure S9. Optimization of the incubation time of FKBP1A-EmGFP with the analyte, FK506. Calibration plots corresponding to 15 min (black ▲), 25 min (red ■) and 35 min (blue ●), followed by 15 min incubation with MB-FK506. Results are shown as mean signals ± the standard error of the mean (n = 3).

Table S4. Performance of the fluororeceptor-based bioassay for quantification of FK506 in whole blood samples (n = 3).

| Nominal concentration (ng mL⁻¹) | Measured concentration (ng mL⁻¹) | RSD (%) | Recovery (%) |
|-------------------------------|---------------------------------|---------|--------------|
| 10                            | 10                              | 5       | 104          |
| 13                            | 14                              | 6       | 109          |
| 16                            | 15                              | 13      | 95           |
| 20                            | 18                              | 9       | 87           |

*RSD, relative standard deviation (n = 3).*
| Method   | Sample material | Sample treatment                                      | LOD/LOQ (ng mL⁻¹) | DR (ng mL⁻¹) | Ref./Manufactor |
|----------|-----------------|-------------------------------------------------------|-------------------|--------------|-----------------|
| ACMIA    | WB              | FK506-carbamate + sonication lysis                    | 0.7/1             | 1–30         | Siemens (Dimension) |
| CEDIA    | WB              | PP: MeOH + ZnSO₄                                      | -/2               | 2–30         | Thermo Scientific |
| CMIA     | WB              | PP: MeOH + ZnSO₄                                      | 0.3/2             | 2–30         | Abbott (ARCHITECT) |
| ECLIA    | WB              | PP: MeOH + ZnSO₄                                      | 0.5/1             | 0.5–40       | Roche (Elecys®)  |
| EMIT     | WB              | PP: MeOH + CuSO₄                                      | -/2               | 2–30         | Siemens (EMIT 2000) |
| QMS      | WB              | PP: MeOH + ZnSO₄                                      | -/0.9             | 1–30         | Thermo Scientific |
| TRFIA    | WB              | Incubation 75 °C, 15 min: Tris-HCl, urea, protease, T20, pH 8 | 0.57/-           | 1.1–28.6     | 9               |
| FIA      | Buffer          | -                                                     | 0.08/-            | 0.15–2       | 10              |
| TIRF     | Buffer          | -                                                     | 0.11/0.57         | -            | 11              |
| ECIS     | S               | -                                                     | 0.17/-            | 1–30         | 12              |
| ACMIA    | WB              | -                                                     | -/3               | 3–31.4       | 13              |
| Paper ELISA | WB            | -                                                     | 1/-               | -            | 14              |
| CBS-MS/MS| WB              | PP: ZnSO₄ (0.1 M):AcN:H₂O (60:30:10, v/v)            | -/1               | 1–50         | 15              |
| PS-MS/MS | DBS             | Extraction: MeOH/CHCl₃/NaOAc (40/60/0.1, v/v)         | 0.2/1.5           | 1.5–30       | 16              |
| Method                | Sample material | Sample treatment                                      | LOD/LOQ (ng mL$^{-1}$) | DR (ng mL$^{-1}$) | Ref./Manufacturer |
|-----------------------|-----------------|-------------------------------------------------------|-------------------------|-------------------|-------------------|
| SPME-MOI-MS/MS        | WB              | PP: AcN/ZnSO$_4$ (0.1 M)/H$_2$O (30/60/10, v/v)      | -/1                     | 1–50              | 17                |
| LC-MS/MS              | DBS (8 mm)      | PP: AcN/ZnSO$_4$                                     | -                       | 1.2–40            | 18                |
| LC-MS/MS              | WB              | 1) Centrifugation                                     | 0.15/0.5                | 0.5–50            | 19                |
|                       |                 | 2) MEPS on-line (C$_8$)                               |                         |                   |                   |
| LC-MS/MS              | DBS (6 mm)      | 1) PP: MeOH and sonication                           | 0.25/1                  | 1–80              | 20                |
|                       |                 | 2) LLE: tert-butil eter                               |                         |                   |                   |
| LC-MS/MS              | Saliva          | 1) Sonication                                        | -/-0.01                 | 0.01–1.6          | 21                |
|                       |                 | 2) PP: AcN                                            |                         |                   |                   |
| Fluorescent           | WB              | PP: MeOH and sonication                              | 3/-                     | 5–70              | This work         |
| receptor-based assay  |                 |                                                       |                         |                   |                   |
|                       |                 |                                                       |                         |                   |                   |

ACMIA: antibody conjugated magnetic immunoassay; CBS: coated blade spray; CEDIA: cloned enzyme donor immunoassay; CMIA: chemiluminescent microparticle immunoassay; DBS: dried blood spot; DR: dynamic range; ECIS: electrochemical immunoassays; ECLIA: electrochemiluminescence immunoassay; EMIT: Enzyme multiplied immunoenzyme technique; FIA: fluorescence immunoassay; LC: liquid chromatography; LLE: liquid-liquid extraction; LOD: limit of detection; LOQ: limit of quantification; MEPS: micro extraction by packed sorbent; MOI: microfluidic open interface; MS/MS: tandem mass spectrometry; PP: protein precipitation; PS: paper spray; QMS: Quantitative Microsphere System; S: serum; SPME: solid-phase microextraction; TIRF: total internal reflection fluorescence; TRFIA: time-resolved fluorescence immunoassay; WB: whole blood.
Table S6. Administered doses of FK506 to the analyzed organ transplanted patients.

| Patient | Administered drug | Dose/24 h |
|---------|-------------------|-----------|
| HCUV1   | Advagraf          | 11 mg     |
| HCUV2   | Advagraf          | 6 mg      |
| HCUV3   | Envarsus          | 4 mg      |
| HCUV4   | Envarsus          | 6 mg      |
| HCUV5   | Advagraf          | 3.5 mg    |
| HCUV6   | Advagraf          | 6.5 mg    |
| HCUV7   | Advagraf          | 2 mg      |
References

(1) Sauerbrey, G. Verwendung von Schwingquarzen zur Wägung dünner Schichten und zur Mikrowägung. Z. Physik 1959, 155 (2), 206–222. https://doi.org/10.1007/BF01337937.

(2) Voinova, M. V.; Rodahl, M.; Jonson, M.; Kasemo, B. Viscoelastic Acoustic Response of Layered Polymer Films at Fluid-Solid Interfaces: Continuum Mechanics Approach. Phys. Scr. 1999, 59 (5), 391. https://doi.org/10.1238/Physica.Regular.059a00391.

(3) Gambinossi, F.; Banchelli, M.; Durand, A.; Berti, D.; Brown, T.; Caminati, G.; Baglioni, P. Modulation of Density and Orientation of Amphiphilic DNA Anchored to Phospholipid Membranes. I. Supported Lipid Bilayers. J. Phys. Chem. B 2010, 114 (21), 7338–7347. https://doi.org/10.1021/jp100730x.

(4) Gambinossi, F.; Lorenzelli, L.; Baglioni, P.; Caminati, G. Silicon Oxide Surface Functionalization by Self-Assembled Nanolayers for Microcantilever Transducers. Colloids Surf. A Physicochem. Eng. Asp 2008, 321 (1), 87–93. https://doi.org/10.1016/j.colsurfa.2008.01.030.

(5) Baltus, R. E.; Carmon, K. S.; Luck, L. A. Quartz Crystal Microbalance (QCM) with Immobilized Protein Receptors: Comparison of Response to Ligand Binding for Direct Protein Immobilization and Protein Attachment via Disulfide Linker. Langmuir 2007, 23 (7), 3880–3885. https://doi.org/10.1021/la0628468.

(6) Ueda, E. K. M.; Gout, P. W.; Morganti, L. Current and Prospective Applications of Metal Ion–Protein Binding. J. Chromatogr. A 2003, 988 (1), 1–23. https://doi.org/10.1016/S0021-9673(02)02057-5.

(7) Raghunath, G.; Dyer, R. B. Kinetics of Histidine-Tagged Protein Association to Nickel-Decorated Liposome Surfaces. Langmuir 2019, 35 (38), 12550–12561. https://doi.org/10.1021/acs.langmuir.9b01700.

(8) Nye, J. A.; Groves, J. T. Kinetic Control of Histidine-Tagged Protein Surface Density on Supported Lipid Bilayers. Langmuir 2008, 24 (8), 4145–4149. https://doi.org/10.1021/la703788h.

(9) Wu, F.-B.; Yang, Y.-Y.; Wang, X.-B.; Wang, Z.; Zhang, W.-W.; Liu, Z.-Y.; Qian, Y.-Q. A Sample Processing Method for Immunoassay of Whole Blood Tacrolimus. Anal. Biochem. 2019, 576, 13–19. https://doi.org/10.1016/j.ab.2019.04.006.

(10) Salis, F.; Descalzo, A. B.; Benito-Peña, E.; Moreno-Bondi, M. C.; Orellana, G. Highly Fluorescent Magnetic Nanobeads with a Remarkable Stokes Shift as Labels for Enhanced Detection in Immunoassays. Small 2018, 14 (20), 1703810. https://doi.org/10.1002/smll.201703810.

(11) Berrettoni, C.; Berneschi, S.; Bernini, R.; Giannetti, A.; Grimaldi, I. A.; Persichetti, G.; Testa, G.; Tombelli, S.; Trono, C.; Baldini, F. Optical Monitoring of Therapeutic Drugs with a Novel Fluorescence-Based POCT Device. Procedia Eng. 2014, 87, 392–395. https://doi.org/10.1016/j.proeng.2014.11.732.

(12) Zhang, Z.; Zhang, Y.; Yu, H.; Rong, S.; Gao, H.; Meng, L.; Dai, J.; Pan, H.; Chang, D. Spherical Carrier Amplification Strategy for Electrochemical Immunosensor Based on Polystyrene-Gold Nanorods @L-Cysteine/MoS2 for
Determination of Tacrolimus. *Talanta* 2020, 220, 121321. https://doi.org/10.1016/j.talanta.2020.121321.

(13) Tempestiti, M.; Di Stasio, E.; Basile, M. R.; Elisei, F.; Antonini, M.; Ettorre, G. M.; Iappelli, M.; Pucillo, L. P. Low Plasma Concentrations of Albumin Influence the Affinity Column–Mediated Immunoassay Method for the Measurement of Tacrolimus in Blood During the Early Period After Liver Transplantation. *Ther. Drug Monit.* 2013, 35 (1), 96–100. https://doi.org/10.1097/FTD.0b013e318279dfd2.

(14) Lantigua, D.; Trimper, J.; Unal, B.; Camci-Unal, G. A New Paper-Based Biosensor for Therapeutic Drug Monitoring. *Lab Chip* 2021, 21 (17), 3289–3297. https://doi.org/10.1039/D1LC00473E.

(15) Rickert, D. A.; Gómez-Ríos, G. A.; Nazdrajić, E.; Tascon, M.; Kulasingam, V.; Pawliszyn, J. B. Evaluation of a Coated Blade Spray-Tandem Mass Spectrometry Assay as a New Tool for the Determination of Immunosuppressive Drugs in Whole Blood. *Anal. Bioanal. Chem.* 2020, 412 (21), 5067–5076. https://doi.org/10.1007/s00216-019-02367-z.

(16) Shi, R.-Z.; El Gierari, E. T. M.; Manicke, N. E.; Faix, J. D. Rapid Measurement of Tacrolimus in Whole Blood by Paper Spray-Tandem Mass Spectrometry (PS-MS/MS). *Clin. Chim. Acta* 2015, 441, 99–104. https://doi.org/10.1016/j.cca.2014.12.022.

(17) Tascon, M.; Alam, Md. N.; Gómez-Ríos, G. A.; Pawliszyn, J. Development of a Microfluidic Open Interface with Flow Isolated Desorption Volume for the Direct Coupling of SPME Devices to Mass Spectrometry. *Anal. Chem.* 2018, 90 (4), 2631–2638. https://doi.org/10.1021/acs.analchem.7b04295.

(18) Sadilkova, K.; Busby, B.; Dickerson, J. A.; Rutledge, J. C.; Jack, R. M. Clinical Validation and Implementation of a Multiplexed Immunosuppressant Assay in Dried Blood Spots by LC–MS/MS. *Clin. Chim. Acta* 2013, 421, 152–156. https://doi.org/10.1016/j.cca.2013.02.009.

(19) Said, R.; Pohanka, A.; Abdel-Rehim, M.; Beck, O. Determination of Four Immunosuppressive Drugs in Whole Blood Using MEPS and LC–MS/MS Allowing Automated Sample Work-up and Analysis. *J. Chromatogr. B* 2012, 897, 42–49. https://doi.org/10.1016/j.jchromb.2012.04.006.

(20) Li, Q.; Cao, D.; Huang, Y.; Xu, H.; Yu, C.; Li, Z. Development and Validation of a Sensitive LC-MS/MS Method for Determination of Tacrolimus on Dried Blood Spots. *Biomed. Chromatogr.* 2013, 27 (3), 327–334. https://doi.org/10.1002/bmc.2795.

(21) Ghareeb, M.; Akhlaghi, F. Development and Validation of a Sensitive and Selective LC–MS/MS Method for Determination of Tacrolimus in Oral Fluids. *J. Chromatogr. B* 2016, 1038, 136–141. https://doi.org/10.1016/j.jchromb.2016.10.008.