High Throughput Homogeneous ADME Assays based on LSPR with Colloidal Gold (SoPRano™)

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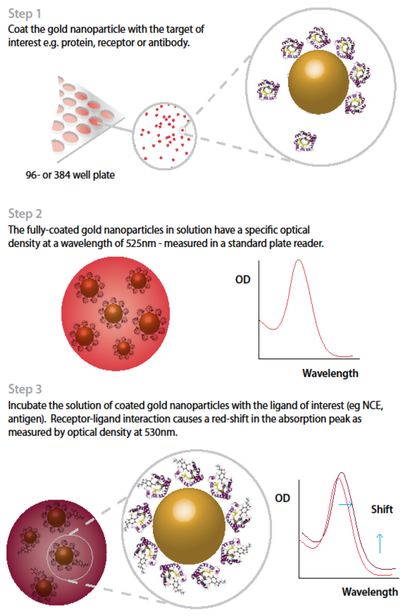
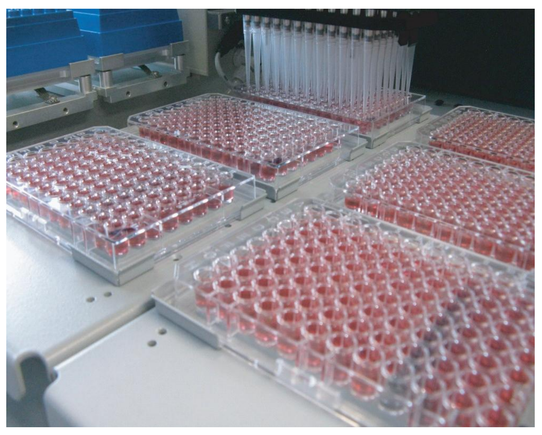
SoPRano™ Technology Platform

- The light absorbance of noble metal nanoparticles at a given wavelength is governed by the refractive index of both the metal and the surrounding medium
- When the nanoparticles are shielded by a biological receptor, its interaction with a ligand increases the RI at the particle surface, which shifts the wavelength of the absorption peak to the red
- Interaction of ligand with receptor can be monitored by measurement of signal at 530nm



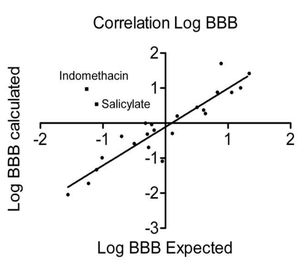
Technology Platform Advantages



- Label-free
- Homogeneous technique: mix and read
- No specialised equipment required:
  - Plate reader to measure signal at 530nm
- Potential for very high throughput
- Robust & reproducible
- Broadly enabling, applicable to various systems:
  - Ligand-receptor/binding
  - Charge/redox interactions
  - Enzymatic reactions
  - Protein conformation
- High sensitivity:
  - pM with proteins
  - Small molecule interactions to <150D (fragment screening)

SoPRano™ Log BBB

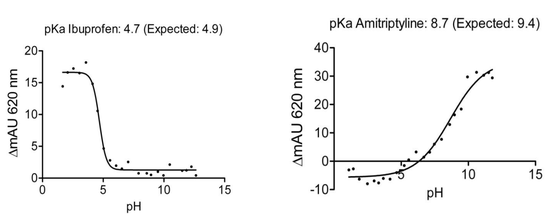
Colloidal nanoparticles are coated with a layer mimicking a lipid bilayer, and then reacted with the drugs at a 100 µM final concentration (Phosphate pH 7.4, 1% DMSO). The interaction of the drugs with the membrane is recorded by the change in OD after ten minutes. Standard curves are made relating the signal recorded to the LogBBB of known drugs. The correlation is valid for passively transported drugs.



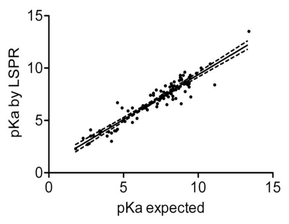
	Calculated
Best-fit values	
Slope	1.102 ± 0.09777
Y-intercept when X=0.0	-0.1103 ± 0.07850
X-intercept when Y=0.0	0.1002
1/slope	0.9079
95% Confidence Intervals	
Slope	0.8975 to 1.305
Y-intercept when X=0.0	-0.2741 to 0.05344
X-intercept when Y=0.0	-0.04876 to 0.2564
Goodness of Fit	
r^2	0.8639
Sy.x	0.3682
Is slope significantly non-zero?	
F	126.9
DFn, DFd	1.000, 20.00
P value	< 0.0001
Deviation from zero?	Significant
Data	
Number of X values	22
Maximum number of Y replicates	1
Total number of values	22
Number of missing values	2

SoPRano™ pKa

- Our technology enables determination of pKa by titering in a pH ladder
- Acidic drugs go from a non-ionized to an ionized state according to pH and basic drugs experience the reverse trend
- Non-ionized or protonated drugs interact with properly designed nanoparticles, although anionic or deprotonated molecules do not as a result of their relative negative charge.



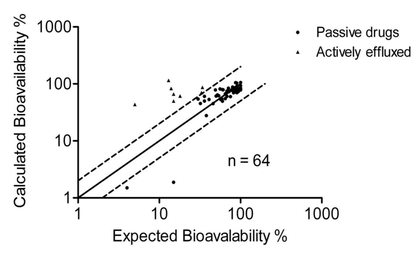
Correlation between the pKa measured by LSPR and data from the literature. The dashed lines show the 95% confidence interval



Best-fit values	
Slope	0.8560 ± 0.03040
Y-intercept when X=0.0	0.8100 ± 0.2269
X-intercept when Y=0.0	-0.9463
1/slope	1.168
95% Confidence Intervals	
Slope	0.7955 to 0.9165
Y-intercept when X=0.0	0.3585 to 1.262
X-intercept when Y=0.0	-1.581 to -0.3924
Goodness of Fit	
r^2	0.8991
Sy.x	0.6694
Is slope significantly non-zero?	
F	793.0
DFn, DFd	1.000, 89.00
P value	< 0.0001
Deviation from zero?	Significant
Data	
Number of X values	91
Maximum number of Y replicates	1
Total number of values	91
Number of missing values	0

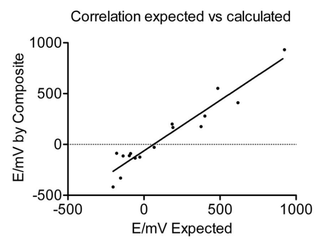
SoPRano™ Bioavailability

Colloidal nanoparticles are coated with a layer mimicking a lipid bilayer, and then reacted with the drugs at a 100 µM final concentration (MES pH 4.5, 1% DMSO). The interaction of the drugs with the membrane is recorded by the change in OD after ten minutes. Standard curves are made relating the signal recorded to the % bioavailability of known acid or basic drugs. Lines on the graph show identity and 2-fold errors.



SoPRano™ Redox Potential

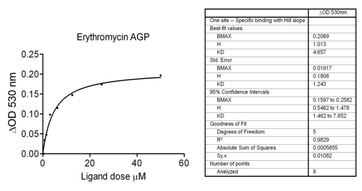
The drug at a given final concentration (50 µM) is introduced in a buffered solution of a metal-conductive polymer composite colloid. After mixing and 10 minutes incubation at room temperature, the optical density is read at two wavelengths and corrected for the blank. A linear relationship is observed between the optical signal and the reduction potential of known molecules, which allows the reduction potential of NCEs to be inferred.



Best-fit values	
Slope	0.9920 ± 0.08027
Y-intercept when X=0.0	-62.59 ± 28.26
X-intercept when Y=0.0	63.10
1/slope	1.008
95% Confidence Intervals	
Slope	0.8198 to 1.164
Y-intercept when X=0.0	-123.2 to -1.970
X-intercept when Y=0.0	2.136 to 119.1
Goodness of Fit	
r^2	0.9160
Sy.x	103.2
Is slope significantly non-zero?	
F	152.7
DFn, DFd	1.000, 14.00
P value	< 0.0001
Deviation from zero?	Significant
Data	
Number of X values	16
Maximum number of Y replicates	1
Total number of values	16
Number of missing values	0

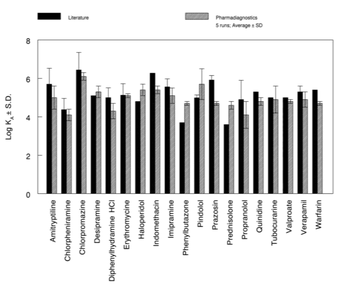
SoPRano™ Plasma Protein-Binding: AGP

Example of Binding isotherm

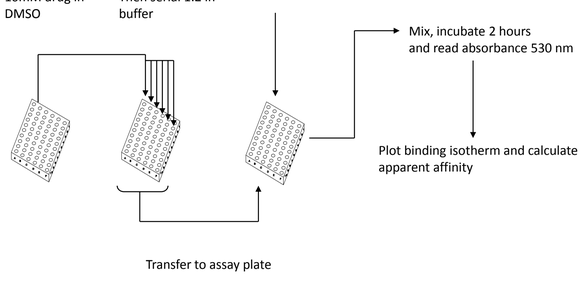


	ΔOD 530nm
One site – Specific binding with Hill slope	
Best-fit values	
BMAX	0.2089
H	1.013
KD	4.657
Std. Error	
BMAX	0.01917
H	0.1808
KD	1.243
95% Confidence Intervals	
BMAX	0.1597 to 0.2582
H	0.8462 to 1.478
KD	1.462 to 7.852
Goodness of Fit	
Degrees of Freedom	5
R^2	0.9829
Absolute Sum of Squares	0.0005855
Sy.x	0.01082
Number of points	
Analyzed	8

Correlation with literature and reproducibility

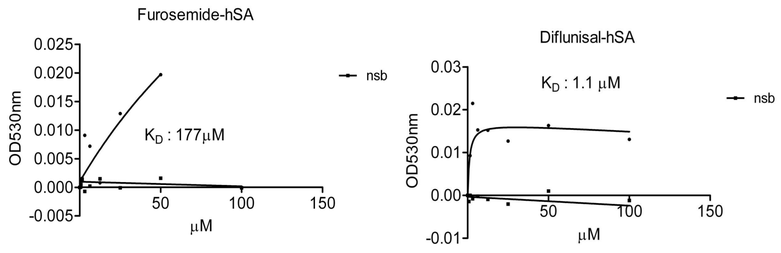


SoPRano™ Plasma Protein-Binding: How it works



SoPRano™ Plasma Protein-Binding: Albumin

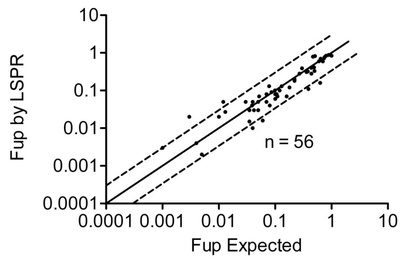
Examples of Binding isotherms



Non-specific binding (nsb) accounts for drug absorbance and DMSO-colloid interaction.

SoPRano™ Plasma Protein-Binding: Free fraction in plasma, Free Fraction in Tissue and Volume of Distribution

Knowing the affinity of a drug for both albumin and AGP further allows to calculate the fraction unbound in plasma (Fup). The lines on the graph indicate identity and 3-fold error. 89% of the data are within the 3-fold error.



We have also developed an algorithm which, by combining the fraction unbound in plasma with pKa and LogD, allows to approximate the fraction unbound in tissue (Fut) and volume of distribution at steady state (VDss). The graphs show identity and 3-fold error. 85% and 75% of the results are within the 3-fold error for Fut and VDss, respectively.