Supplementary information

Heterogeneous kinetics in the functionalization of single plasmonic nanoparticles

Matěj Horáček\textsuperscript{1,2}, Rachel E. Armstrong\textsuperscript{1,2}, and Peter Zijlstra\textsuperscript{1,2}

\textsuperscript{1}Molecular Biosensing for Medical Diagnostics, Faculty of Applied Physics, Eindhoven University of Technology, P.O. Box 513, 5600 MB, Eindhoven, The Netherlands

\textsuperscript{2}Institute for Complex Molecular Systems, Eindhoven University of Technology, P.O. Box 513, 5600 MB, Eindhoven, The Netherlands

Table of contents

| Topic                                                                 | Page |
|----------------------------------------------------------------------|------|
| Sample preparation                                                   | S2   |
| Single particle detection and recognition (hyperspectral microscopy)| S3   |
| pH, ionic strength and Debye length in citrate buffer                | S5   |
| Nanoparticles (TEM analysis)                                          | S6   |
| Core-shell Mie-Gans model                                             | S7   |
| Measured bulk refractive-index sensitivity                            | S9   |
| Heterogeneity in the end-point plasmon shift                         | S10  |
| Gamma distribution fit                                                | S12  |
| Kinetics results for 0 M NaCl                                         | S13  |
| Heterogeneity in the initial binding rates                            | S14  |
| References                                                            | S16  |
Sample preparation

The CTAB concentration in the suspension of nanorods was first lowered to 1 mM, and the concentration of the particles was adjusted to yield a coverage of 300 – 400 particles in the 130 x 130 µm² field-of-view of the microscope.

A 30 µL drop of the suspension was then spincoated on a coverslip at 2000 rpm for 90 seconds. To ensure that the particles are firmly attached to the substrate, the coverslips were first functionalized with (3-Mercaptopropyl)trimethoxysilane (MPTMS). For thiolation we used the following protocol:

1. Clean coverslips by sonication in methanol for 20 mins.
2. UV/ozone clean coverslips for 90 mins.
3. Immerse coverslips in 5% v/v solution of MPTS in methanol for 3 minutes.
4. Rinse coverslips with methanol, then clean by sonication in methanol for 20 mins.

After the last wash in methanol the nanorods were immediately spincoated onto the coverslips. After spincoating the coverslips were rinsed with 1M NaCl solution, PBS, MQ water, ethanol, methanol, and dried in N₂ flow. This ensures that the CTAB is rinsed off the surface of the particles. The coverslips with particles were typically used within 2 weeks of preparation.
Single particle detection and recognition (hyperspectral microscopy)

White-light scattering spectra were recorded for all particles in the field-of-view at the same time using wide field hyperspectral microscopy. Individual nanorods are characterized by a narrow Lorentzian spectrum, allowing us to discard clusters based on the lineshape and linewidth of the spectrum.

To record white-light spectra we a fiber-coupled laser-driven xenon white-light source (Energetiq). A series of 18 nm bandpass filters with center wavelengths ranging from 670 nm to 890 nm are sequentially introduced in the emission path of microscope, and a wide field image is captured for each center-wavelength (EM gain 5, integration time 500 ms). The scattering spectrum can then be reconstructed by determining the detected scattered intensity for each individual particle for each center wavelength. This method is fast for large numbers of particles, as the data acquisition is reduced to capturing few images only, i.e. one per center wavelength.

The intensity scattered by the particle is calculated from the images by fitting each diffraction-limited spot in the image with a 2D Gaussian:

\[ f(x, y) = I_{bg} + A \exp \left[ -\left( \frac{(x-x_0)^2}{2\sigma_x^2} + \frac{(y-y_0)^2}{2\sigma_y^2} \right) \right], \quad \text{Eqn. S1} \]

where \( I_{bg} \) is the background intensity in the image, \( x_0 \) and \( y_0 \) are the coordinates of the 2D Gaussian, \( \sigma_x \) and \( \sigma_y \) are the standard-deviations in the x and the y-direction, and \( A \) is the amplitude of the Gaussian. In the fitting procedure we assumed a symmetrical spot, i.e. \( \sigma_x = \sigma_y \). The total detected intensity is then given by the volume under the Gaussian as

\[ I_{det} = 2\pi A\sigma_x^2. \quad \text{Eqn. S2} \]

To recover the scattering spectrum we correct \( I_{NP} \) for the optical response of the setup by normalizing to the experimentally determined (non-zero) background signal for each center wavelength:

\[ I_{NP} = \frac{I_{det}}{I_{bg}}. \quad \text{Eqn. S3} \]

A few examples of scattering spectra of individual gold nanorods are shown in the main text in Fig. 1c. We observe single and narrow Lorentzian spectra which linewidth falls within the range of 110 ± 40 meV indicating single particles. In Fig. S1 we also show examples of the scattering spectrum of clusters of nanoparticles, which exhibit a double peak (blue and green curves) or no clear peak feature at all (red curve). These clusters can easily be distinguished from the spectra of individual particles and are discarded from the further data analysis.

We determined the measurement error of the hyperspectral microscopy by measuring spectra of individual single gold nanorods repetitively. Gold nanorods were functionalized on clean glass coverslip and the measurement was performed in the flow cell in MQ water. Our setup performs with an accuracy of 0.5 nm in the plasmon wavelength and 3 meV in the plasmon linewidth determination.
Figure S1: Three examples of scattering spectra of clusters found on the sample. The examples of scattering spectra of individual single gold nanorods are shown in the main text in figure 1c.
pH, ionic strength and Debye length in citrate buffer

All experiments were performed in citric acid buffer of 100 mM strength. Citric acid buffer consists of two components: citric acid and trisodium citrate, the ratio of which determines the pH. We performed our experiments in a broad range of buffer pH, and since citrate molecules are deprotonated as pH increases, it is necessary to carefully evaluate the ionic strength of the buffer [1]. The acid dissociation constants ($pK_a$) determine at which pH value the deprotonation of the acidic group occurs. The (de)protonation of the citrate molecule directly influences the ionic strength of the buffer (Fig. S2a) and therefore the corresponding Debye length (Fig. S2b). Moreover, the citrate present in the citric acid buffer coats the surface of the gold nanoparticles [2] and therefore induces a negative surface charge on the particles [3]. The $pK_a$ values for the three acid groups in citrate are listed in Table S1.

| chemical        | $pK_a$  |
|-----------------|---------|
| citric acid     | 3.14    |
|                 | 4.77    |
|                 | 6.40    |

Table S1: Acid dissociation constants ($pK_a$) of the three acid groups in citrate [11].

Figure S2: (a) Ionic strength of 100 mM citric acid buffer with no additional NaCl, (b) Debye length for 0 M and 1 M of additional salt as a function of buffer pH.
Nanoparticles (TEM analysis)

We used commercially available colloidal nanorods from Nanoseedz in Hong Kong (product # NR-25-780). The nanorods are in aqueous solution and are stabilized by cetyltrimethylammonium bromide (CTAB). They exhibit an ensemble-average longitudinal plasmon at 780 nm.

We characterized the geometrical properties of our nanorods by transmission electron microscopy (TEM) (Fig. S3a). We found a distribution of diameter of $19 \pm 3$ nm and length of $69 \pm 7$ nm (Fig. S3b) giving dispersions of $\pm 15.8\%$ and $\pm 10.1\%$, respectively. Therefore, the underlying distributions of aspect ratio in our sample is $3.7 \pm 0.7$ and volumes $19014 \pm 6365$ nm$^3$ (Fig. S3c) giving dispersions of $\pm 18.9\%$ and $\pm 33.3\%$, respectively.

**Figure S3:** (a) TEM image of our sample of gold nanorods dropcast on a carbon-coated copper grid. (b) Histograms of diameters and lengths of our nanorods as measured using TEM. (c) Histogram of aspect ratios of our nanorod sample and in the inset a histogram of the particles’ volumes.
Core-shell Mie-Gans model

A small metal nanoparticle in an electromagnetic field can be approximated as an oscillating dipole. This results in absorption and scattering of the incident light that can be highly enhanced when a plasmon resonance is excited. Both, the scattering and the absorption, can be relatively simply calculated for particles much smaller than the wavelength of the incident light by Mie-Gans theory.

Due to the binding of molecules the refractive index surrounding the gold nanorod changes, inducing a shift of the plasmon resonance. We assume an effective medium approach in which these molecules establish a shell of finite thickness around the nanorod. The metallic nanoparticle surrounded by a shell can be modelled using a modified Mie-Gans theory that treats core-shell particles [4]. The material of the nanoparticle is determined by the dielectric function $\varepsilon(\omega)$, where Johnson and Christy’s data for gold are used, and the particle is immersed in water with a frequency-independent dielectric function $\varepsilon_m = 1.77$. The shell is modelled as an ellipsoid extending the core metallic nanoparticle characterized by its thickness $s$ and frequency-independent dielectric function $\varepsilon_s$. The polarizability of the core-shell metallic ellipsoid’s longitudinal plasmon is [4]:

$$ \alpha(\omega) = \varepsilon_0 V_p \left( \frac{(\varepsilon_s - \varepsilon_m) \left[ \varepsilon_s + (\varepsilon(\omega) - \varepsilon_s) \left( L_p - \frac{V_p}{V_s} L_s \right) \right] + \frac{V_p}{V_s} \varepsilon_s (\varepsilon(\omega) - \varepsilon_s)}{\varepsilon_s + (\varepsilon(\omega) - \varepsilon_s) \left[ L_p - \frac{V_p}{V_s} L_s \right] + \frac{V_p}{V_s} L_s \varepsilon_s (\varepsilon(\omega) - \varepsilon_s)} \right) $$ , Eqn. S4

with $L_p$ the depolarization factor of the metallic particle core and $L_s$ the depolarization factor of the whole particle, which are both characterized by their own eccentricities. $V_p$ is the volume of the metallic nanoparticle core and $V_s$ is the volume of the shell spheroid.

Calculation of expected distribution of plasmon shifts

The magenta histogram in Figure 3b in the main text is calculated by evaluating the scattering cross section $\sigma_{\text{sca}} = k^4|\alpha|^2 / 6\pi\varepsilon_0^2$ for a set of particle-dimensions ($n = 215$) extracted from TEM measurements. By taking the exact dimensions found in TEM images we estimate the contribution of the size dispersion to the plasmon shift.

We calculated the expected shift by considering an index matched shell ($\varepsilon_s = 1.77$), and subsequently a shell with an increased dielectric function due to the presence of ssDNA. The thickness of the shell $s$ was determined by treating the ssDNA molecule as a worm-like chain [5] giving $s = 11$ nm for our 50 nt sequence. The dielectric function of the shell is used as a fitting parameter to match the experimental results. We find an only slightly increased dielectric function for the shell ($\varepsilon_s = 1.90$), which indicates a hydrated and non-close-packed ssDNA shell. In line with the experiments, only particles that exhibit a plasmon wavelength in the range of 670 nm – 890 nm are analyzed. Note that this effective medium approach neglects any higher order corrections originating from heterogeneity in ssDNA conformation.
Calculation of the expected distribution of initial rates

The magenta histogram of initial rates shown in Figure 6b in the main text is calculated by again evaluating the scattering spectra for particles immersed in water with an empty shell, followed by calculation of the spectra of the same particle with a shell with dielectric function \( \varepsilon_s = 1.80 \). These two spectra are then assigned to time \( t = 0 \) s and \( t = 1 \) s, respectively, and the rate at which the plasmon shifts is then extracted in the same way as in the experiment (see main text), however here only from two data points. This approach takes into account that the rate at which the plasmon shifts depends on the geometry of the particle due to variations in refractive index sensitivity. Note that this yields a distribution of normalized rate constants that are then multiplied by a constant to match the average measured rate constant.

Limitations of the model

However, some remarks should be made for applying the core-shell Mie-Gans model to model the optical properties of the gold nanorods and the binding events. The model holds only for particles much smaller than the wavelength of light, equivalent to a sphere of approximately 50 nm in diameter. The nanorods which are used in the experiments have dimensions around (70 nm - 20 nm), having a volume smaller than a sphere of 60 nm diameter. Furthermore, the rods are approximated by ellipsoids, which causes deviations of their scattering cross sections due to both the shape approximation and the reduction in volume of particles. Moreover, the nanorods in the experiments are immobilized on a glass coverslip, thus ssDNA cannot bind to the bottom of the nanorod. Because of those deviations the absolute plasmon peak positions as well as the absolute scattering cross sections will differ from the experiments. However, the plasmon shifts and their distribution will be comparable to the experimental situation.
Measured bulk refractive-index sensitivity

We measured the bulk refractive-index sensitivity of our gold nanorod. The spectra of substrate immobilized gold nanorods were measured in media of different refractive indices prepared by mixing of MQ water \((n_{MQ} = 1.329 \,[6])\) with ethylene glycol \((n_{EG} = 1.427 \,[7])\) in different ratios. The bulk refractive-index sensitivity is defined as \(S_\lambda = \frac{d\lambda_{SP}}{dn}\), where \(d\lambda_{SP}\) is the change of the plasmon wavelength caused by a change of refractive index \(dn\) of the surrounding media \([8]\). The recorded data for bulk sensitivity together with values calculated using Mie-Gans theory are shown in Figure S4. We find absolute values lower than the theoretical predictions due to the presence of the substrate in our experiments that shields part of the probe volume of the particles. To further analyze the particle-to-particle differences in the bulk sensitivity we evaluated the sub-population of nanoparticles characterized by plasmon wavelengths of 750 nm - 800 nm (see dashed lines in figure S4). We find coefficients of variation of 8 % and 6 % for the theoretical and experimental data, respectively. This implies that the heterogeneity in sensitivity is dominated by the presence of different aspect ratios in the sample, and not by different distances and orientations of the particles with respect to the substrate.

Figure S4: The bulk refractive index sensitivity of gold nanorods determined experimentally and using Mie-Gans model. By considering the sub-population of nanoparticles characterized by plasmon wavelengths in the range 750 - 800 nm we extracted \(CV_{Mie} = 8\%\) and \(CV_{exp} = 6\%\).
**Heterogeneity in the end-point plasmon shift**

We observe a large scatter which can be partly ascribed to the effect of different particle volumes and/or differences in ssDNA coverage. Therefore, we apply the above described core-shell Mie-Gans model and analyze different contributions to the measured variance in plasmon shift.

We assume all variables are normally distributed. This allows us to establish different contributions to the heterogeneity as a convolution of the individual Gaussian distributions, i.e. the variances are additive. We can therefore write:

\[
\sigma_{tot}^2 = \sigma_{vol}^2 + \sigma_{cov}^2 ,
\]

where \( \sigma_{tot}^2 \) represents the total (measured) variance of the distribution, \( \sigma_{vol}^2 \) represents the variance caused by the distribution of particle volumes in our sample (note that the distribution of aspect ratios does not play a role as we select the subpopulation of particles with a plasmon resonance between 750 nm and 800 nm), and \( \sigma_{cov}^2 \) represents the contribution from variations in the ssDNA coverage of the particles.

The latter can again be split into two contributions, the first arising from the minimal variance expected from the Poissonian distributed number of strands per particle (\( \sigma_{Pois}^2 \)), and the second from variations in the average number of ssDNA per particle (\( \sigma_{DNA}^2 \)). In other words

\[
\sigma_{cov}^2 = \sigma_{Pois}^2 + \sigma_{DNA}^2 .
\]

We estimate \( \sigma_{Pois}^2 \) by assuming a typical footprint of 10 nm\(^2\) per molecule, in combination with an average particle surface area of 4000 nm\(^2\). This results in an average of \( \mu = 400 \) strands per particle. Assuming a Poissonian distribution this yields a standard deviation of \( \sigma_{Pois} = \sqrt{\mu} = 20 \). The coefficient of variation is thus \( CV = \frac{\sigma}{\mu} = 5 \% \). We assume that this CV translates directly to a CV of the plasmon shift, in other words we assume that the average shift per molecule is a constant. This gives a variance in the plasmon shift that is given by \( \sigma_{Pois}^2 = (\Delta S \times CV)^2 = 0.5 \text{ nm}^2 \) for ideal conditions (i.e. pH 1.7 and 1 M of NaCl), which is the value we used in the main text. The resulting variances for all measurements are listed in Table S2.

| sample | \( \mu \) (nm) | \( \sigma_{tot}^2 \) (nm\(^2\)) | \( \sigma_{vol}^2 \) (nm\(^2\)) | \( \sigma_{cov}^2 \) (nm\(^2\)) | \( \sigma_{Pois}^2 \) (nm\(^2\)) | \( \sigma_{DNA}^2 \) (nm\(^2\)) | CV\(_{DNA}\) (%) |
|--------|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|----------------|
| pH 1.7 [NaCl] = 1M S1 | 14.7 | 5.2 | 0.9 | 4.3 | 0.5 | 3.8 | 13 |
| pH 1.7 [NaCl] = 1M S2 | 17.4 | 5.3 | 1.2 | 4.0 | 0.8 | 3.3 | 10 |
| pH 3 [NaCl] = 1M | 11.5 | 4.3 | 0.5 | 3.8 | 0.3 | 3.5 | 16 |
| pH 5 [NaCl] = 1M | 3.32 | 1.12 | 0.04 | 1.08 | 0.03 | 1.05 | 31 |

*Table S2: Heterogeneity in the end-point plasmon shift for data from Figure S5 and Figure 3b from the main text.*
Figure S5: (left) Correlations of end-point plasmon shifts with the plasmon wavelength for different pH of buffer. For all samples we find a positive correlation characterized by the shown $R^2$ of the linear fit, however significant heterogeneity is still present. (right) Histograms of plasmon shifts of the sub-population characterized by plasmon wavelengths in the range 750 - 800 nm, their dispersion is highlighted by the variance of the distributions. On top of them the Mie-Gans model histograms (magenta) calculated for particle sizes measured by TEM. This calculated heterogeneity corresponds only to effect of size distribution and is also expressed in terms of the variance of the distribution. The remaining heterogeneity is attributed to particle-to-particle variations in ssDNA coverage.
Gamma distribution fit

Here we compare the ability of different models to fit the timetraces. As can be seen in Figure S6a, a single exponential using 3 fit parameters $\rho$ clearly does not fit well, whereas a double exponential ($\rho = 4$) captures the data better. However, there is no reason to assume that two well-defined rates are dominant in the coating process, it is more likely instead that a continuous distribution of rates is present. We therefore also fitted the data with a stretched exponential ($\rho = 3$) and a Gamma distribution of exponentials ($\rho = 3$), both of which yield analytical equations suitable for direct fitting to the timetrace (see main text). Figure S6b summarizes the accuracy of fits as a distributions of $R^2$ from individual timetraces. For the Gamma distribution we find slightly better fits represented by higher $R^2$ values, which is why we choose this model to fit our data in Figure 6 in the main text.

Figure S6: (a) A typical example of a timetrace of ssDNA binding and corresponding fits using one exponential, two exponentials, stretched exponential and Gamma distribution of exponentials. (b) Accuracy of fits to recorded timetraces of individual particles upon ssDNA binding characterized by Coefficient of determination $R^2$. The box corresponds to distribution 25th to 75th percentile quartile, the flat line is the median and error bars represent the full distribution.
**Kinetics results for 0 M NaCl**

We performed measurements of dynamic plasmon shifts of single particles in response to binding of ssDNA at pH 1.7, 3, and 5, and for 0 M NaCl added to the citric acid buffer. We fitted the timetraces of individual particles with Eqn. 5, to extract initial kinetic binding rates $k_0$. In line with results for 1 M of additional NaCl (Fig. 7a) we found heterogeneous log-normally distributed binding rates, which magnitude decreases with increasing pH (Fig. S7).

![Graph](image)

**Figure S7:** Distributions of initial binding rates as a function of buffer pH at 0 M of additional NaCl. Here, the scatter corresponds to the mean and of the distribution, and error bars to the standard deviation of the distribution.
Heterogeneity in the initial binding rates

We analyze different contributions to the observed heterogeneous kinetics of ssDNA binding to gold nanorods (Fig. 6b in the main text and Fig. S8) for multiple ssDNA concentrations. First we analyze the dispersion of particle sizes present in our sample of nanorods. In contrast with the end-point measurements we find no correlation of initial rates with the plasmon wavelength over all probed concentrations and chemical conditions. This is further supported by no correlation found when our core-shell Mie-Gans model is applied. This implies that the particle-size distribution is not the dominant factor in determining the observed heterogeneity.

To further probe the origin of the kinetic heterogeneity we now focus on different contributions to the variance in initial rates. We again assume all variables are normally distributed. This allows us to establish different contributions to the heterogeneity as a convolution of the individual Gaussian distributions, i.e. the variances are additive. We can therefore write:

$$\sigma_{tot}^2 = \sigma_{pcle}^2 + \sigma_{bar}^2 ,$$

Eqn. S7

where $\sigma_{tot}^2$ represents the total (measured) variance of the distribution, $\sigma_{pcle}^2$ represents the variance caused by the distribution of particle volumes in our sample (determined by the core-shell Mie-Gans model), and $\sigma_{bar}^2$ represents all other contributions. As outlined in the main text, we attribute $\sigma_{bar}^2$ to a distribution of particle surface-charges which results in a distribution of energy barriers that have to be overcome by the ssDNA in order to reach the particle surface. The spread in initial rates due to the distribution of energy barriers is then determined from the coefficient of variation of the distribution $CV_{bar} = \sigma_{bar}/\Delta SP$. The results are summarized in Table S3, where we find that the initial rate exhibits due to a spread in energy barriers a typical $CV_{bar}$ of 30%.

| sample          | $\mu$ (s$^{-1}$) | $\sigma_{tot}^2$ (s$^{-2}$) | $\sigma_{pcle}^2$ (s$^{-2}$) | $\sigma_{bar}^2$ (s$^{-2}$) | $CV_{bar}$ (%) |
|-----------------|------------------|-----------------------------|-----------------------------|-----------------------------|----------------|
| [ssDNA] = 2 μM  | 5.9E-04          | 4.4E-08                     | 1.4E-09                     | 2.4E-08                     | 26             |
| [ssDNA] = 250 nM| 9.4E-05          | 7.2E-10                     | 5.1E-11                     | 6.6E-10                     | 27             |
| [ssDNA] = 25 nM | 3.3E-06          | 2.0E-12                     | 1.1E-13                     | 1.8E-12                     | 41             |

Table S3: Heterogeneity in the initial rates analysis for data from Figure S7.
Figure S8: (left) Correlations of initial rates with plasmon wavelength of the nanoparticles for different ssDNA concentrations. We find no clear correlation consistent across the 10 samples we measured. The initial rates obtained using the core-shell Mie-Gans model (bottom graph) again corresponds only to the effect of the size distribution of our sample. (right) Histograms of initial binding rates $k_0$, their dispersion is highlighted by the variance of the distributions. On top of them the Mie-Gans model histograms (magenta) calculated for particle sizes measured by TEM. The additional spread in experimental data of approximately 30% we ascribe to particle-to-particle variations in the surface charge resulting in a range of energy barriers. All images are on the same scale (1.4 decade on y-axis).
References

[1] M. Borkowski, "ChemBuddy.com," 2005. [Online]. Available: http://www.chembuddy.com. [Accessed 26 9 2017].

[2] J.-W. Park and J. S. Shumaker-Parry, "Structural Study of Citrate Layers on Gold Nanoparticles: Role of Intermolecular Interactions in Stabilizing Nanoparticles," J. Am. Chem. Soc., vol. 136, no. 5, p. 1907–1921, 2014.

[3] S. H. Brewer, W. R. Glomm, M. C. Johnson, M. K. Knag and S. Franzen, "Probing BSA Binding to Citrate-Coated Gold Nanoparticles and Surfaces," Langmuir, vol. 21, no. 20, p. 9303–9307, 2005.

[4] M. Guyot-Sionnest and P. Guyot-Sionnest, "Synthesis and Optical Characterization of Au/Ag Core/Shell Nanorods," J. Phys. Chem. B, vol. 108, no. 19, p. 5882–5888, 2004.

[5] M. Doi and S. F. Edwards, The theory of Polymer Dynamics, Oxford: Clarendon Press, 1988.

[6] S. Kedenburg, M. Vieweg, T. Gissibl and H. Giessen, "Linear refractive index and absorption measurements of nonlinear optical liquids in the visible and near-infrared spectral region," Optical Materials Express, vol. 2, no. 11, pp. 1588-1611, 2012.

[7] E. Sani and A. Dell'Oro, "Optical constants of ethylene glycol over an extremely wide spectral range," Optical Materials, vol. 37, pp. 36-41, 2014.

[8] J. Becker, A. Trügler, A. Jakab, U. Hohenester and C. Sönnichsen, "The Optimal Aspect Ratio of Gold Nanorods for Plasmonic Bio-sensing," Plasmonics, vol. 5, no. 2, p. 161–167, 2010.

[9] W. D. Kumler and J. J. Eiler, "The Acid Strength of Mono and Diesters of Phosphoric Acid. The n-Alkyl Esters from Methyl to Butyl, the Esters of Biological Importance, and the Natural Guanidine Phosphoric Acids," J. Am. Chem. Soc., vol. 65, no. 12, p. 2355–2361, 1943.

[10] V. Verdolino, R. Cammi, B. H. Munk and H. B. Schlegel, "Calculation of pKa Values of Nucleobases and the Guanine Oxidation Products Guanidinoxydantoin and Spiroiminodihydantoin using Density Functional Theory and a Polarizable Continuum Model," J. Phys. Chem. B, vol. 112, no. 51, p. 16860–16873, 2008.

[11] R. B. Martin, "A complete ionization scheme for citric acid," J. Phys. Chem., vol. 65, no. 11, p. 2053–2055, 1961.