Langmuir
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Molecular Recognition by Contact Angle: Proof of Concept with DNA Hybridization

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Received December 22, 2008. Revised Manuscript Received March 4, 2009

A molecular recognition reaction supported by a solid-phase assay drives a specific change in the solid−solution interfacial tension. This prompts contact angle (CA) analysis, being a straightforward route to evaluate this property, to play the inedited role of label-free probe of the reaction. The concept is proven by the successful recognition of DNA hybridization and is further supported by the agreement between the results and the underpinning thermodynamics.

Introduction

Recognition and quantification of biomolecular interactions pervade the basic life sciences, spanning cell physiology, disease diagnostics, and drug discovery. Molecular recognition is achieved by free-solution or solid-phase essays, exploiting a reporter label or a measurable physicochemical transformation univocally related to the recognition event (label-free techniques).[1] Because of their unique advantages, solid-phase methods such as fluorescence microarrays and surface plasmon resonance (SPR) biosensors[2] have gained a prominent role in molecular recognition. However, refinement of the existing methods and development of new ones, seeking for higher overall performance, remain key open issues.

From a thermodynamics standpoint, at equilibrium a ligand−receptor binding reaction confined at the interface between the solid that bears the receptors and the solution that supplies the ligands is described by the (surface) van't Hoff isotherm[3]

$$\Delta_r G^0 = -\frac{\Delta\gamma}{\Gamma_{LR}} - RT \ln K \qquad (1)$$

where $\Delta_r G^0$ is the change in the standard molar Gibbs free energy of the reaction in free solution, $\Delta\gamma$ is the variation of the solid−solution interfacial tension upon binding, Γ_{LR} is the surface density of the ligand−receptor complexes, and K is the equilibrium constant (or binding affinity). In other words, eq 1 indicates that the free energy released after the molecular recognition event drives a specific change in the solid−solution interfacial tension and in turn it exerts an additional surface stress on the solid.[3]

This phenomenon has recently found a breakthrough application in cantilever biosensors, which provide label-free, energy-based transduction of the binding reaction by balancing the stress change with a readable nanomechanical response.[3−7] However, it is well known that solid−liquid interfacial tension can be directly measured by contact angle, CA, analysis, which is perhaps the top classic technique for the investigation of surfaces,[8] prompting the abduction that CA might play the unedited role of label-free probe of ligand−receptor binding. The term inedited is used because CA and related tensiometric techniques are extensively used to study unspecific protein adsorption[9] and were very recently applied to study streptavidin−biotin interaction,[10] but they were never explicitly proposed and tested in molecular recognition experiments.

When a droplet is placed onto a solid surface, it reaches equilibrium with the surface and the surroundings under the action of the interfacial tension at the contact line at which drop, surface, and surroundings meet, forming a definite CA. CA is linked to the interfacial tension by the Young−Dupré equation (see also Results and Discussion section and ref 8.). The transposition of this familiar physicochemical phenomenon to molecular recognition is sketched in Figure 1. Here the surface, phase S, is functionalized with a receptor, the droplet, phase B, is a solution of unspecific and specific ligands with respect to that receptor (panels a and b, respectively), and C is the surroundings phase.

In view of eq 1, specific binding featured by system b makes a specific contribution to the solid−solution interfacial tension, γ_{SB}, that is missed in the interfacial tension of the unspecific system (a), γ^0_{SB}. Binding is thus univocally

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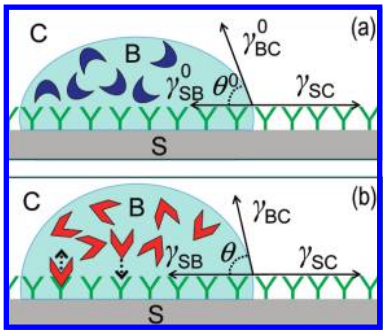

Figure 1. Schematic of sessile drop CA systems at equilibrium formed by a drop of ligand solution (phase B), a receptor-functionalized surface (phase S), and a surrounding phase (phase C); the CA is indicated by θ. Panel a represents the unspecific case, where ligands and receptors do not bind. Panel b represents the specific case, where ligands and receptors bind.

associated to the differential of the solid−solution interfacial tensions of the two systems, $\Delta\gamma_{SB}$. If the solution−surroundings interfacial tensions of the control and specific systems, γ^0_{BC} and γ_{BC}, are equal or not remarkably different (as often applies for molecular recognition systems, see the Results and Discussion section) then this also translates into different CAs, θ and θ^0. Analogous concepts hold if pure buffer is considered instead of the unspecific ligand solution.

In the following text, this working principle will be tested and discussed for a familiar example of molecular recognition: detection of DNA hybridization (duplex formation), i.e., specific DNA binding, supported by gold-coated silicon wafers.

Materials and Methods

Gold-coated ($7.5 \times 7.5\,mm^2$) silicon wafers (SPI supplies, West Chester, PA) were cleaned with piranha solution (3/1 w/w H_2SO_4/H_2O_2) for 20 min. After accurate rinsing with Milli-Q water, a 30 μL drop of a 5 μM buffered solution (PBS 10 mM) of 5′-thiol-modified ssDNA probes (Invitrogen) was deposited onto each substrate. The substrates were then placed for 36 h at room temperature in an uncovered storage box placed in a sealed chamber saturated with NaCl. This protocol to prepare a ssDNA self-assembled monolayer (SAM) onto gold surfaces is a widely used and investigated procedure; under the adopted conditions a surface coverage of about 10^{13} molecules per cm^2 is sorted (see, for example, ref 11).

The adopted DNA sequences are commonly used for animal species identification. The probe ssDNA sequence is a 35 bp probe for the sheep COI region (GTC CTA ACT GTA GAC CTA ACC ACA ACA AAC CTA GA). The ssDNA complementary to the probe, the ssDNA 50% complementary to the probe (specific for the chicken COI region), and the noncomplementary ssDNA have sequences of TCT AGG TTT GTT GTG GTT AGG TCT ACA GTT AGG AC, TCG ATA TTA GTG GCA GTT AAT TCG GGT TGG AGT AC, and TTA ATC GTT GAA CAA ACG AAC C. They were used at a concentration of 1 μM in PBS 10 mM at 25 °C. Under these experimental conditions, they have binding energies with the probe ssDNA of $-53.7\ kcal\cdot mol^{-1}$ (duplex), $-2.8\ kcal\cdot mol^{-1}$ (dimer), and $-0.2\ kcal\cdot mol^{-1}$ (dimer), respectively (calculations performed with Vector NTI Advance 10, by InVitrogen).

All of the used chemicals were from Sigma-Aldrich, Germany.

Sessile drop CA analysis and pendant drop experiments[8] were conducted on a DSA 10 Mk2 device from Kruss, Germany, equipped with a controlled atmosphere chamber and the data processed by Drop Profile Analysis software, also by Kruss. All of the measurements were performed in the room atmosphere at 25 °C.

Results and Discussion

Onto different wafers functionalized with the same probe ssDNA and immersed in cyclohexane (at 25 °C) we placed droplets (volume < 1 μL) of pure buffer and of 1 μM buffered solutions of noncomplementary ssDNA, of 50% complementary ssDNA, and of complementary ssDNA. Cyclohexane was preferred to air as the surrounding fluid because it prevents the droplets from evaporating during the attainment of equilibrium and sorts higher CA values. The droplet profiles were acquired by a video camera (sample images are given in Figure 3) and were used to extract the CAs.

Droplet spreading kinetics (Figure 2) indicate that the overall equilibrium with the substrate was reached by any drop about 600 s after the deposition. However, at the adopted conditions, the chemical equilibrium for the duplex formation reaction is attained in about 300 s.[6,12] By merging this information, we assumed that after 600 s both mechanical and chemical equilibria were established and therefore CA values were extracted from the droplet profiles collected after this period. Shapes of all drops were fitted with a circular function. This function is fast and robust and gives accurate results for droplets with volumes lower than 1 μL.[13]

The mean CA values after five independent replicates, displayed in Figure 3, resulted: $\theta^b = (30.3 \pm 3.4)°$, $\theta^0 = (30.3 \pm 3.0)°$, $\theta^{0.5} = (47.9 \pm 2.0)°$, $\theta^1 = (55.6 \pm 2.2)°$, where the superscripts b, 0, 0.5, and 1 refer to the buffer droplet, to the noncomplementary ssDNA droplet, to the 50% complementary ssDNA droplet, and to the complementary ssDNA droplet, respectively; the errors are the SD of the mean. θ^1 is significantly higher than the CAs of the buffer droplet and of the control droplets, θ^b, θ^0, and $\theta^{0.5}$, thus unambiguously recognizing DNA duplex formation.

The CA is linked to the interfacial tension by the Young−Dupré equation,[8] which expresses the equilibrium of the drop under the action of the interfacial tensions at the contact line between the three involved interfaces. The Young−Dupré equations for the specific and unspecific systems represented in panels a and b of Figure 1 read $\gamma^0_{SB} + \gamma^0_{BC}\cos\theta^0 = \gamma_{SC}$ and $\gamma_{SB} + \gamma_{BC}\cos\theta = \gamma_{SC}$, respectively, where the subscripts refer to the phases forming the interface. By equating γ_{SC}, one obtains the relationship among γ^0_{SB}, γ_{SB}, θ^0, and θ

$$\Delta\gamma^0_{SB} = \gamma^0_{BC}\cos\theta^0 - \gamma_{BC}\cos\theta \qquad (2)$$

where $\Delta\gamma^0_{SB} = \gamma_{SB} - \gamma^0_{SB}$ is the differential of the solid−solution interfacial tensions between the specific and the unspecific systems that appear in eq 1, viz., the thermodynamic parameter sensitive to specific binding.

Now, the droplet−cyclohexane interfacial tensions, according to the above introduced notations hereafter referred to as γ^b_{BC}, γ^0_{BC}, $\gamma^{0.5}_{BC}$, and γ^1_{BC}, were determined through the pendant drop method.[8] Thus resulted in $\gamma^b_{BC} = (40.97 \pm 1.15)\ mN\cdot m^{-1}$, $\gamma^0_{BC} = (39.22 \pm 0.92)\ mN\cdot m^{-1}$, $\gamma^{0.5}_{BC} = (37.70 \pm 1.20)\ mN\cdot m^{-1}$, and $\gamma^1_{BC} = (37.33 \pm 0.52)\ mN\cdot m^{-1}$. As expected, because

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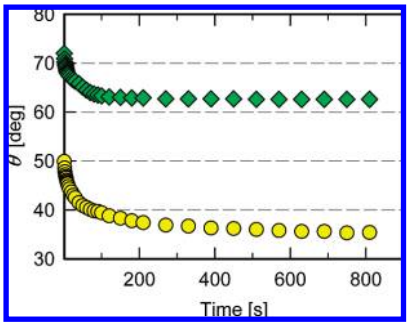

Figure 2. Spreading kinetics of droplets of pure buffer (circles) and of a 1 μM buffered solution of complementary ssDNA (diamonds).

of the very similar solvation properties of the complementary and 50% complementary ssDNA molecules, $\gamma_{BC}^{0.5}$ and γ_{BC}^{1} are equal. For analogous reasons, γ_{BC}^{0} is only slightly different. Also, since the ssDNA solutions are very dilute, γ_{BC}^{b} is not much higher than γ_{BC}^{0}, $\gamma_{BC}^{0.5}$, and γ_{BC}^{1} (less than 10%). Under these conditions and in view of the Young−Dupré equation and eqs 1 and 2, the CA is directly linked to the solid−solution interfacial tension, and thus it is sensitive to both specific binding and binding free energy $\Delta_r G^0$. This explains the results reported in Figure 3, where the CA of the noncomplementary ssDNA ($\Delta_r G^0$ = −0.2 kcal·mol^{-1}) is equal to the CA of the pure buffer, followed by the CA of the 50% complementary ssDNA ($\Delta_r G^0$ = −2.8 kcal·mol^{-1}) and the CA of the complementary ssDNA ($\Delta_r G^0$ = −53.7 kcal·mol^{-1}). Finally, by properly substituting into eq 2 the experimental values of γ_{BC}^{b}, γ_{BC}^{0}, $\gamma_{BC}^{0.5}$, and γ_{BC}^{1} and θ^{b}, θ^{0}, $\theta^{0.5}$, and θ^{1} reported above, one can calculate the differential of the solid−solution interfacial tensions between complementary ssDNA and buffer, noncomplementary ssDNA, and 50% complementary ssDNA. The result is $\Delta\gamma_{SB}^{b}$ = (13.7 ± 2.8) mNm^{-1}, $\Delta\gamma_{SB}^{0}$ = (12.3 ± 2.6) mNm^{-1}, and $\Delta\gamma_{SB}^{0.5}$ = (4.2 ± 1.8) mNm^{-1}, respectively. It is worth noticing that these values, representing the differentials of the interactions of the targets with experimentally identical SAMs of probes, rule out the contribution from nonspecific molecular interactions as well as other background contributions.

As expected,[3] the surface tension differentials are consistent with the surface stress applied by DNA duplex formation evaluated with microcantilevers, which ranges from 2 to 15 mN·m^{-1}.[4,6,12,14] From one side, this is a valuable cross-check of the detection of DNA hybridization by CA; from the other side, it is an additional hint that the surface van't Hoff isotherm (eq 1) is an accurate thermodynamic model to describe ligand−receptor interactions confined at solid−solution interfaces.

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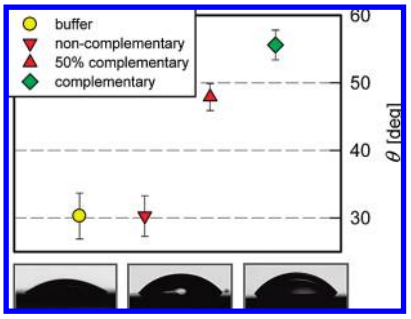

Figure 3. CA, indicated by θ, of droplets of pure buffer (circles), of a 1 μM buffered solution of noncomplementary ssDNA (down triangles), of a 1 μM buffered solution of 50% complementary ssDNA (up triangle), and of complementary ssDNA (diamonds). Each θ point is the mean value of five independent replicates; the error bars refer to the SD of the mean. θ values were extracted from the profiles of the droplets 10 min after deposition, when both mechanical and chemical equilibria held. The images below the panel are representative examples of sessile drops and refer from left to right to a buffer droplet, to a 50% complementary ssDNA droplet, and to a complementary ssDNA droplet, respectively.

Conclusions

The results of the inedited and successful application of CA analysis in the detection of DNA duplex formation were presented and discussed.

From the point of view of the physical chemistry of surfaces, they constitute an additional independent proof of the validity of the surface van't Hoff isotherm, that until now was exclusively verified against data from nanomechanical cantilever experiments.[3,14] From the application side, they give a first proof that CA may play the role of label-free probe of receptor−ligand interactions.

Indeed, the presented application of CA analysis sounds appealing. Compared to the biosensors on the scene, CA is inherently easier access and cheaper.[15] It is directly based on the transduction of the binding free energy, thus it might be interesting for the detection of low-molecular-weight species or in an affinity determination. Furthermore, the recent development of picoliter instruments (down to 100 pL droplets for a contact diameter of 70 μm)[13] makes possible applications in which small volumes of analyte are mandatory and/or multiplexed operation is advantageous. In view of these desirables implementations, experiments focused on the determination of the effective analytical performances of CA analysis on "field" trials (DNA hybridization as well as other ligand−receptor interactions) are needed and are currently underway.

Acknowledgment. We thank Raffaele Mazza and Paolo Ajmone Marsan for providing the biochemical reagents and Ivano Alessandri, Paolo Colombi, Stefania Mitola, Patrizia Dell'Era, Pietro Poesio, Marco Rusnati, and Marco Presta for fruitful feedback, discussions, and encouragement.

(15) A custom CA apparatus can be set up for less than $5000.