Insulin Delivery Gel

DOI: 10.1002/ange.201106252

# A Synthetic Approach Toward a Self-Regulated Insulin Delivery System**

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Stimuli-responsive "smart gels" that undergo physicochemical property changes in response to external stimuli can provide both a functional and structural basis for self-regulated materials and systems. Of particular interest are those materials sensitive to chemical stimuli, that is, the fluctuation in the concentration of specific biomolecules, that can mimic biofeedback systems, thus providing many attractive platforms for biomedical applications. Glucose has been a molecule of interest for decades owing to its relevance to the treatment of diabetes. Diabetes is not an infectious disease, but its rapidly increasing worldwide prevalence has been recognized as a pandemic, and thus diabetes poses a serious global health threat much like epidemics of truly infectious diseases, such as HIV/AIDS.[1] Despite the necessity for the continuous and accurate glycemic control in the management of insulin-dependent diabetes mellitus (IDDM), the current palliative treatment relies almost solely on the patients self injection of insulin; this self injection not only impinges on the quality of life of the patient but also fails to precisely control the dose of insulin, where an overdose must be strictly avoided as it causes serious hypoglycemia.

For the preparation of self-regulated insulin delivery systems, the following two approaches have historically prevailed.[2] One is based on enzymatic reactions between glucose oxidase (GOD) and glucose, a similar rationale to those exploited in commercial glucose sensors. The other type utilizes carbohydrate-binding lectin proteins, such as concanavalin A (Con A), as a complementary binder to glucose. These protein-based materials eventually denature and lose activity, and thus are not practical for long-term use and storage. Another problem is the strong cytotoxicity of Con A; this cytotoxicity has, thus far, prevented any clinical applications of these materials.

Herein we describe a totally synthetic alternative to these materials. Phenylboronic acid (PBA), a synthetic molecule capable of reversibly binding with 1,2- or 1,3-*cis*-diols including glucose, is utilized as the molecular basis.[3] Our previous studies have shown that the glucose-dependent shift in the equilibrium of PBA between the uncharged and anionically charged forms, when coupled with a properly amphiphilic three-dimensional backbone (or gel), could induce a reversible change in the volume of the gel.[4] The resultant abrupt and rapid change in the hydration, under certain conditions, could cause a localized dehydration of the gel surface, that is, a so-called skin layer, thus offering a method to instantly control the permeation of gel-loaded insulin.[4a,c]

This goal is a great challenge and relies on achieving sufficient glucose sensitivity under physiological pH and temperature, that is, pH 7.4 and 37 °C, while also fine tuning the system so it shows a gated response to the change in glucose concentration critically at the level of normoglycemia, i.e., ca. 1 $g_L^{-1}$. To achieve this goal, we have systemically explored the structure–property correlation by focusing on (meth)acrylamide-based hydrogels. The major efforts have been directed to controlling the apparent $pK_a$ of PBA; the $pK_a$ depends not only on chemical structure of PBA, but also on the state of hydration.[4e] To make the situation more complicated, in a hydrogel environment the degree of hydration is a function of (as well as temperature, ionic strength, pH, and sugar concentration) the hydrophilicity, rigidity, and density of the main chain components and the amount of PBA. Herein we describe a gel that meets all the above criteria; the chemical structure of this gel turned out to be a remarkably simple copolymer system. A smart gel has been shown to act as an artificial pancreas under conditions closely related to human glucose homeostasis.

As illustrated in Figure 1 a, PBA derivatives in water exist in equilibrium between uncharged (i) and anionically charged (ii) forms. Upon addition of glucose, only the charged PBA (ii) can form a stable complex with glucose (iii), and the formation of this complex results in an apparent decrease in the amount of anionically charged PBA (ii). On consumption of the phenylboronate (ii), the equilibrium between (i) and (ii) shifts toward the latter (ii). Since the complexed PBA (iii) is also anionically charged, further addition of glucose leads to a shift in the equilibrium (i + ii + iii) toward the phenylboronate anions (ii + iii), and a decrease in the amount of

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[**] This research is supported by the Japan Science and Technology Agency (JST), Core Research of Evolutional Science & Technology (CREST), and, in part, by the Japan Society for the Promotion of Science (JSPS) through the "Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program)," initiated by the Council for Science and Technology Policy (CSTP).
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201106252.

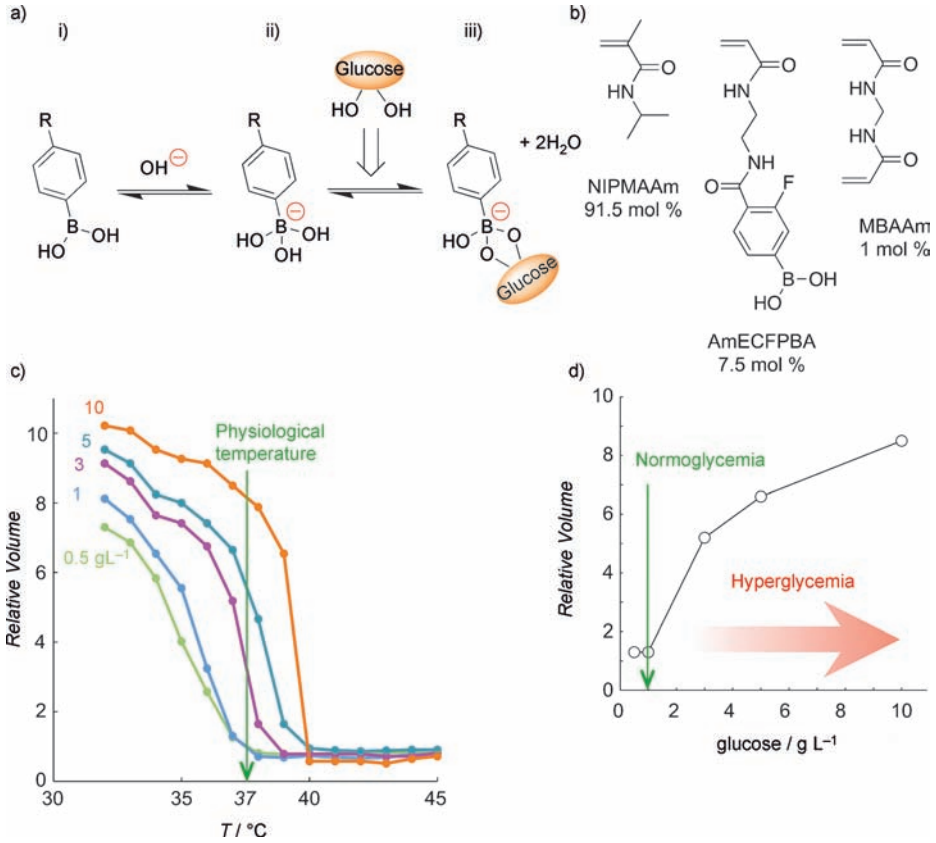


***Figure 1.*** a) Glucose-dependent equilibria of phenylboronic acid. b) Structures of the monomers contained in the gel and their optimized molar amounts in the feed solution so as to obtain glucose sensitivity under physiological pH and temperature (pH 7.4 and 37 °C). c) Phase diagram of the gel showing the equilibrium volume changes as a function of temperature for various glucose concentrations investigated at pH 7.4. The green arrow indicates physiological temperature (37 °C). d) Volume change of the gel as a function of the glucose concentration at 37 °C. The green arrow indicates the normoglycemic concentration of glucose (1 $g\,L^{-1}$).

glucose shifts the equilibrium toward the uncharged phenylboronate (Figure 1 a). Such a glucose-dependent change in the phenylboronate equilibrium, when coupled with an amphiphilic three-dimensional polymeric backbone (or gel), gives rise to a reversible volume change (change in hydration) of the gel in response to a change in glucose concentration.[4] This volume change, termed volume phase transition, is primarily driven by the change in the counterions' osmotic pressure in the gel synchronized with the equilibrium shift of PBA.

In our effort to find a system that is sufficiently glucose sensitive under physiological pH and temperature (pH 7.4 and 37 °C) a primary challenge stemed from the relatively high intrinsic $pK_a$ of PBA, which is typically around 9 (see the Supporting Information, Scheme S2). Under the physiological pH (7.4) the weak acidity of PBA results in a limited fraction of charged phenylboronate, which is responsible for the interaction with glucose. A strategy to decrease the $pK_a$ of PBA has been reported previously.[4b,e] Briefly, it involves the introduction of strongly electron-withdrawing substituents to the phenyl ring.[5] Such a structural change results in an electron-poor boron atom of PBA, thus making it more acidic (i.e. a lower $pK_a$); the magnitude of this effect is dependent on both the structure and position of the substituents (see the Supporting Information, Scheme S2).[6] Accordingly, a new derivative 4-(2-acrylamidoethylcarbamoyl)-3-fluorophenylboronic acid (AmECFPBA), possessing *para*-carbamoyl and *meta*-fluoro substituents, was synthesized (the Supporting Information; Scheme S1). Although it is often the case that strongly electron-withdrawing substituents (representatively, fluorocarbons) are very hydrophobic, thus limiting the use of the resultant compounds in an aqueous environment, the combination of the two substituents in the new derivative could minimize such a side effect. The $pK_a$ of the new derivative was determined to be 7.2 (see the Supporting Information, Figure S1), a value appreciably lower than physiological pH (7.4), thus suggesting glucose sensitivity under such a condition. Figure 1 b and c show structure and phase diagrams (obtained at pH 7.4), respectively, of a copolymer gel with a poly(*N*-isopropylmethacrylamide) (PNIPMAAm) main chain and bearing an optimized (in terms of obtaining the glucose sensitivity under physiological conditions) molar content of AmECFPBA (0.075 molar fraction of the total amount of monomer in the feed solution; see also the Supporting Information, Figure S2). It is shown in Figure 1 c that the gel shrinks with an increase of the temperature as a result of the thermo-sensitivity of the PNIPMAAm main chain. When observed at constant temperatures the gel becomes more swollen with an increase in the amount of glucose owing to the increased fraction of pheneylboronate anions, as mentioned above. Separately shown in Figure 1 d (extracted from Figure 1 c) is the volume change of the gel as a function of the glucose concentration under physiological pH and temperature (pH 7.4 and 37 °C). Notably, at physiological temperature the gel is completely shrunken (dehydrated) for a glucose concentration of up to 1 $g\,L^{-1}$ (a value corresponding to normoglycemia), whereas with any further increase in glucose concentration it becomes markedly swollen (hydrated). These observations thus demonstrate the ability of the gel to undergo a volume phase transition in response to a change in glucose concentration, the threshold value of which is in agreement with that of normoglycemia (1 $g\,L^{-1}$) under physiological conditions (pH 7.4, 150 mM NaCl, 37 °C). Importantly, a 0.075 molar fraction of AmECFPBA was critical to achieve the desirable glucose sensitivity as obtained

  

in Figure 1d. That is to say, any further increase in the fraction of AmECFPBA, although leading to a more significant glucose-dependent volume change, caused an excessive decrease in the hydrophilicity of the overall polymer network owing to the strong hydrophobicity of AmECFPBA (see the Supporting Information Figure S2), thus making the gel insensitive under the physiological temperature. Such precision in the structure control would not have been realistic without the use of (ready-to-copolymerise) PBA-containing monomers, as opposed to strategies involving polymeric state reactions.

In addition to the above-disclosed states of equilibrium, (only) when the magnitude of the change in hydration is substantial, the gel can also exhibit a metastable phase known as a skin layer by a process of shrinkage (dehydration).[7] Note that none of the other materials (including those in our previous reports) have shown such an abrupt response that would be sufficient to induce the skin layer under physiological conditions. A skin layer is a thin surface layer of a collapsed (dehydrated) polymer network, which, once formed, upon a decrease in the glucose concentration, inhibits both further penetration of glucose and further loss of water.[4a,c] As a result, two coexistent phases, that is, an inner hydrated and a surrounding dehydrated phase, can stably prevail for an appreciably long period of time, during which permeation of any preloaded molecules such as insulin can also be suppressed. Our previous study showed that a skin layer formed on a micro gel bead composed of a poly(*N*-isopropylacrylamide) main chain and 3-acrylamidophenylboronic acid could be maintained for at least 10 hours (before further shinking occurred), despite its relatively small size (several hundred μM in diameter) and a large change in glucose concentration (from 5 $g L^{-1}$ to 0 $g L^{-1}$), although it was observable only under nonphysiological conditions, such as pH 9 at 28 °C.[4c] As already mentioned, our strategy to achieve the controlled release of insulin capitalizes on the skin layer. Figure 2 summarizes kinetic observations of the skin layer that elaborates upon switching the glucose concentration between normo- (1 $g L^{-1}$) and hyperglycemic (2 $g L^{-1}$) levels. In order to visualize the dynamic and reversible process of dehydration that is distinctive for the skin layer, the fluorescence reporter agent 8-anilino-1-naphthalenesulfonic acid (ANS) was utilized for its ability to increase fluorescence intensity when exposed to an environment of decreased local polarity (implicated in dehydration). Figure 2a shows both transmittance (left) and fluorescence (right) top-view images of the gel that had been initially equilibrated in hyperglycemic conditions (2 $g L^{-1}$) and then transferred, back and forth, to normoglycemic conditions (1 $g L^{-1}$); the prompt and reversible kinetics of the skin layer can be seen as changes in the intensity of the blue color of ANS (see also the Supporting Information, Figure S3). When the gel was kept under normoglycemic conditions (1 $g L^{-1}$) for 24 hours, a slight decrease in volume was observed but not complete shrinkage; this result supports the preservation of the skin layer, and thus the partitioning between the inner-hydrated and the outer-dehydrated phases, even after 24 hours. Figure 2b shows cross-sectional profiles of the ANS fluorescence intensity during the growth of the skin layer. These profiles reveal a progressive increase in the density of the collapsed (dehydrated) polymer chains; this increase in density becomes more prominent over time and at the vicinity of the edge (surface) of the gel, a feature consistent with the

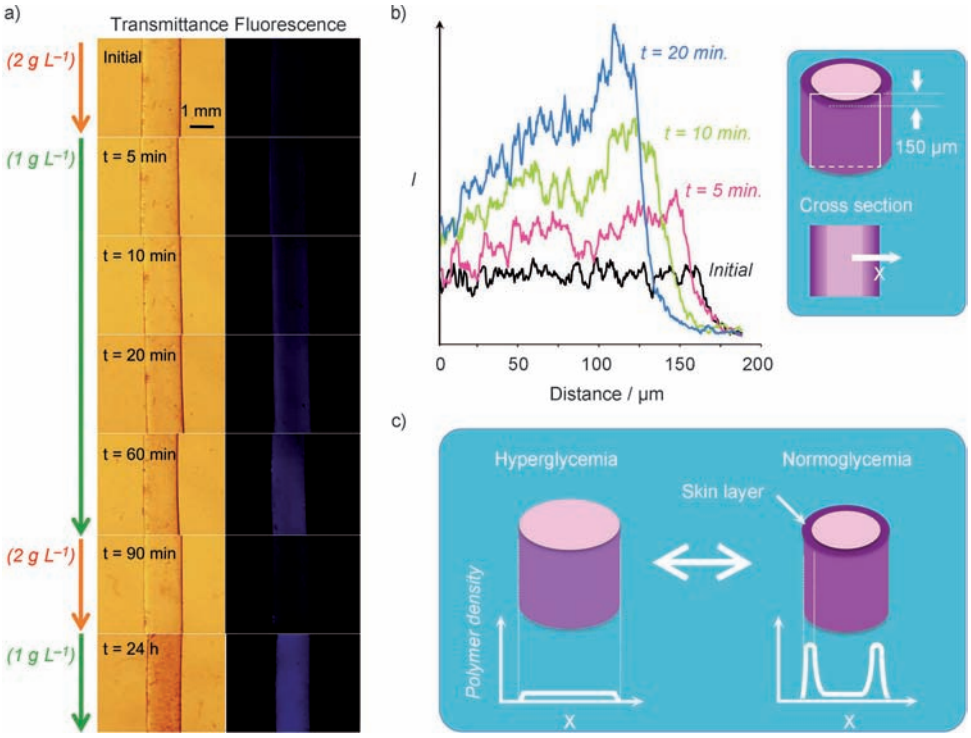


***Figure 2.*** a) Time-course (left) transmittance and (right) 8-anilino-1-naphthalene sulfonic acid (ANS) fluorescence top-view images of a cylinder-shaped piece of gel when changing the glucose concentration under pH 7.4 and 37 °C. Indicated in each transmittance image are the elapsed times after the initial lowering of the concentration from 2 $g L^{-1}$ to 1 $g L^{-1}$. In the fluorescence images, the appearance of the skin layer is seen as an increase in the intensity of the blue color of ANS, thus correlating with the dehydration on the gel surface. Conversely, a reduction in the intensity upon an increase of the glucose concentration ($t = 90$ min.) is indicative of the disappearance of the skin layer. b) Changes in the ANS intensity profiles of a 150 μm depth cross-section (from the gel surface) during the growth of the skin layer. c) Schematic representation of the cross-section density distribution of the polymer network in the gel when reversibly forming the skin layer.

formation of a skin layer. These observations are schematically presented in Figure 2c.

FITC-labeled (bovine) insulin was loaded onto the gel by first allowing the gel to be hydrated (swollen) in a solution containing insulin and then introducing the skin layer onto its surface. Figure 3a–c show time-course profiles of the released insulin under different addition patterns of glucose under physiological conditions. It is clearly demonstrated that the release of insulin from the gel can be continuously controlled

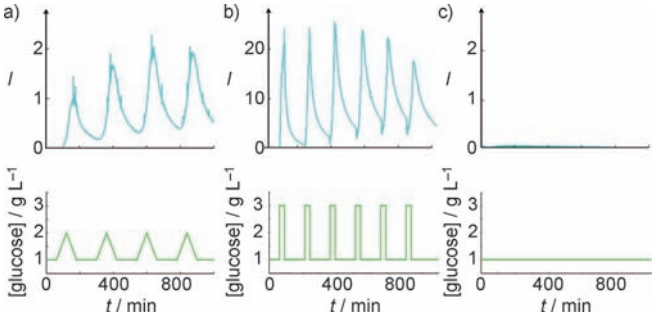


***Figure 3.*** (Top) Time-course changes in the fluorescence intensity of FITC-labeled bovine insulin released from the gel under physiological conditions (pH 7.4, $I = 0.15$, 37 °C). (Bottom) Temporal patterns of the fluctuation in glucose concentration, investigated in each experiment.

by the skin layer (see also the Supporting Information, Figure S4) with close correspondence to each addition pattern of glucose. In Figure 3a, the glucose concentration was varied between 1 $g L^{-1}$ and 2 $g L^{-1}$, which correspond to normoglycemia and the typical cut-off value for the diagnosis of diabetes, respectively. Note that the profile in Figure 3b, for which the glucose concentration is increased to up to 3 $g L^{-1}$, is displayed at 10 times the scale as for Figure 3a and Figure 3c. By comparing these spectra, it can be determined that under such more severe hyperglycemic conditions the rate of release is 10 times greater (Figure 3b; 3 $g L^{-1}$) as compared to the other glucose concentrations (Figure 3a; 2 $g L^{-1}$). On the other hand, under normoglycemic conditions (shown in Figure 3c) the release is effectively halted over the timescale of a day (at least 1000 min). Overall, sharp onsets of the release in response to increases in glucose concentration are readily achievable, whereas during the offset processes, that is upon decreased glucose concentration, somewhat substantial induction times are seen depending on the addition pattern of glucose. This delayed shut-off response can be attributed to a leakage of insulin through the skin layer at the premature stage of the polymer-network contraction. Based on the fact that the diffusion coefficient of a solute molecule in a gel is dependent on the density of the polymer network, that is, effective mesh size relative to the size of solute molecule, in the present case insulin,[8] we anticipated that the leakage could be reduced by increasing the density of the cross-linker (*N*,*N*′-methylene-bis(acrylamide)) or the total concentration of monomers at preparation (with consideration to the molecular weight of insulin). Figure 4 verifies the validity of such a remedy. For this particular experiment, the gel was prepared with twice as high a polymer-network density (monomer concentration at preparation: 3 M) as compared to the previous experiments (1.5 M). As expected, the leakage of insulin became barely visible owing to the increased network density or suppressed diffusion of insulin, thus reducing the induction times. This observation is of critical importance to prevent overdosing of insulin once a normoglycemic condition is reached. Remarkably enough, this gel did not allow leakage of insulin for a few days while normoglycemia was maintained, and upon re-increase of the glucose concentration, the gel did allow a burst release of insulin with no visible lag time (see the Supporting Information, Figure S5). Neither deterioration in the shape of the gel nor cracks were observed after repeated formation of the skin layer over two weeks. Also worthy of mention is that the normal pattern of physiological response to a stepwise increase in blood glucose concentration is initially a rapid and pulsatile release of insulin followed by a ramp in secretion.[9] Indeed, this type of pattern could also be imitated by lowering the amount of insulin remaining in the gel (see the Supporting Information Figure S4).

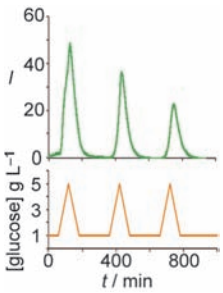


***Figure 4.*** Improved insulin release using a gel with a dense polymer network with twice the monomer concentration compared to Figure 3. (Top) Time-course change in fluorescence intensity of FITC-labeled bovine insulin released from the gel under physiological aqueous conditions. (Bottom) Temporal pattern of the glucose concentration investigated in the experiment.

Our results demonstrate that a nonprotein-based, totally synthetic smart gel can achieve "lasting" control over the provision of insulin under conditions closely associated with human glucose homeostasis. Owing to the significant rate and magnitude of the glucose-induced phase transition, the gel can promptly form a skin layer, thus providing a rationale for the surface-controlled provision of insulin. This non-equilibrium mechanism leads to a remarkably shortened response time (owing to the short diffusion distance across the skin layer: typically on the order of 100 μm); this shortened response time is an important criterion for the tight control of the insulin dosage. Such a surface-limited mechanism (as opposed to more bulk-dependent systems) in theory allows the prediction of the release profile, independent of the size and topological structure of the gel. This feature may facilitate ease and accuracy in the management of the administration of insulin. Furthermore, the size independency may lead to compatibility with other well-developed technologies such as dwelling needles and semi-embedded devices. Aside from the skin-layer-based strategy described herein, the material can also be exploited as other mechanically functional elements such as chemical valves or pumps for related applications.[10] In addition, with its ability to cause dramatic changes to other parameters including size, charge density, conductivity, and permittivity (owing to the change in hydration), the material provides a unique platform for electrochemical sensors and actuators. There is also room for size control (e.g., preparation in micro- or nanoporous

  

structures which are already well established),[11] through which the response of the gel would be further improved.[12] Further efforts are directed towards these possibilities along with in vivo studies.

## Experimental Section

Gels were prepared by radical copolymerization in a 1 mm diameter glass capillary using 2,2′-azobisisobutyronitrile (AIBN) as an initiator in the presence of *N*,*N*′-methylene-bis(acrylamide) (MBAAm) as a cross-linking agent in DMSO. The concentrations of the total monomers, AIBN, and MBAAm in feed were 1.5 M, 7.5 mM, and 15 mM, respectively. The reaction was carried out at 60 °C for 24 h. The obtained gels were taken out of the capillaries and washed with an excess amount of DMSO and distilled water to remove any unreacted reagents and to thoroughly exchange the solvent from DMSO to water. Diameter changes of the capillary gels were observed under an optical microscope (SZX16, OLYMPAS, Japan). To control the temperature, a thermostated water-flow chamber was utilized on the microscope stage, to which the gel capillaries (pieces of 5 mm length in the shrunken state) were placed with each experimental buffer solution. The equilibrium volume changes when decreasing the temperature were recorded under various glucose concentrations to obtain the phase diagrams. Based on the cylindrical symmetry of the gel, the degree of swelling (relative volume) of the gel was defined as $(d/d_0)^3$, where $d_0$ is the diameter obtained at 45 °C (with no glucose) whereas $d$ is those observed under various conditions.

More detailed methods for material preparation, optimization, analysis of the skin layer, release experiment along with p$K_a$ data of various PBAs as determined by titration, additional data of skin layer observation, and the release experiments are provided in Supporting Information.

Received: September 4, 2011
Published online: December 9, 2011

**Keywords:** gels · insulin · phenylboronates · polymers · skin layer

[1] United Nations Resolution 61/225: World Diabetes Day (from 83rd plenary meeting, 20 December **2006**).

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