Biomimetic glass nanopores employing aptamer gates responsive to a small molecule†

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We report the preparation of 20 and 65 nm radii glass nanopores whose surface is modified with DNA aptamers controlling the molecular transport through the nanopores in response to small molecule binding.

Single synthetic nanopores are of interest as potential mimics of biological pores such as gated ion channels. Controlled transport through these synthetic pores can be achieved by integrating polymers responsive to chemical or physical stimuli.[1] Stimuli reported to date include pH,[2] ionic strength, temperature,[3] UV light as well as recognition of an ion,[4] a small molecule[5] or a protein.[6] The responsive components in the system can exhibit a single or a combination of several responses to the environmental conditions, such as changes in charge and conformation of surface-bound macromolecules. Here we significantly broaden the range of molecular effectors that can be employed to control the gating of such channels *via* the use of aptamers, DNA or RNA molecules selected for their ability to bind to, and fold in response to, specific aqueous targets, as the gating macromolecule.[7]

As the substrate onto which our gating aptamers have been grafted we have employed a single glass nanopore electrode (Fig. 1, see ESI† for preparation details).[8] It consists of a platinum microdisk electrode embedded at the bottom of a conical nanopore made in glass, with the circular orifice of the pore having dimensions in the range 5 to 100 nm. Redox-active molecules diffuse and migrate through the orifice connecting the bulk solution and nanopore interior. The deep cone shape of the nanopore results in an important transport characteristic: the steady-state flux of molecules into the pore is limited by the resistive restriction at the pore orifice and is independent of other geometrical parameters of the pore. Earlier, we have reported that surface modification of the glass nanopore electrodes with spiropyran moieties leads to the photochemical control of molecular transport through the pore orifice.[9] Specifically, we were able to electrostatically control the diffusion of a positively charged species by UV light-induced conversion of the neutral surface-bound spiropyran to its protonated merocyanine form, and restore the transport through the pore orifice by irradiation of the electrode with visible light to reverse the spiropyran transformation. The present work introduces our proof-of-principle experiments where biomimetic glass nanopores are prepared with transport properties responsive to small molecules as a result of introducing aptamers as the sterically gating macromolecules.

Critical to our investigation, aptamers can be engineered to undergo a large-scale conformational response to specific small molecules.[7a,b,10] For example, the aptamer **A1** (Table 1) employed in the present work and based on a 32-base sequence reported by Stajonovic *et al.*, binds and folds in response to cocaine.[10] Specifically, the aptamer remains partially unfolded in the absence of a target but forms a three-way junction when cocaine binds to an internal cavity (Fig. 2). In order to optimize the extent to which aptamers can gate a multi-nanometre diameter pores, we have expanded the length scale over which the aptamers undergo binding-induced folding using two modified variants of **A1**, namely **A2** and **A3** (Table 1).

We expected that the cocaine-induced folding of an aptamer in the nanopore orifice would cause an increase in the rate of diffusion through the nanopore as a result of an increase in the free volume contained within. Thus, by covalently attaching cocaine-binding aptamers to the surface of the nanopore

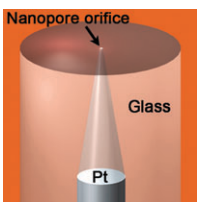

Fig. 1 Schematic representation of the glass nanopore electrode.

Table 1 Aptamer sequences (5′ → 3′)

A1	AGACAAGGAAAATCCTTCAATGAAGTGGGTCG
A2	AGACAAGGAAAA-T_{60}-TCCTTCAATGAAGTGGGTCG-MB
A3	$(AGACAAGGAAAATCCTTCAATGAAGTGTGGGTCG)_3$-MB

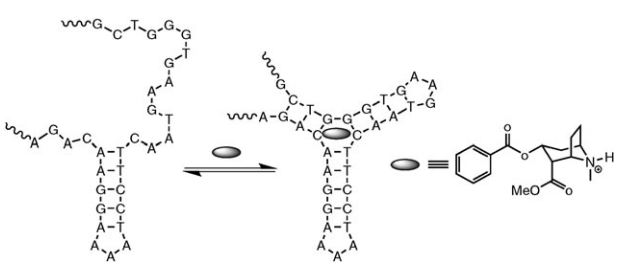

Fig. 2 Conformational change of aptamer in response to cocaine binding.

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electrode we expected to create a nanopore that mimics a protein channel in which molecular transport is selectively controlled in response to small molecule binding. The present report serves as a proof of principle that single glass nanopores can be used as a platform for creating small molecule-responsive biomimetic nanopores.

To attach the aptamers to the glass nanopores, we elected to modify the nanopore surface with amines,[9] followed by a bis-functional cross-linker containing maleimide and *N*-hydroxy-succinimide.[11] The sulfhydryl-reactive maleimide should activate the glass surface for reaction with a free thiol groups incorporated in the aptamer during its synthesis.

In order to verify and optimize our surface modification procedure, we employed silica nanoparticles as a proxy, assuming that their surface chemistry is similar to that of the glass nanopores. We modified 290 nm diameter silica spheres, prepared using the Stöber method,[12] with amines using $(EtO)_3Si(CH_2)_3NH_2$, followed by treatment with succinimidyl 4-[*N*-maleimidomethyl]-cyclohexane-1-carboxylate (SMCC) in DMF in the presence of DMAP (Scheme 1). This approach was adapted from the previously reported work on the activation of silica with maleimide.[13] Silica nanoparticles were then treated with the thiol-terminated **A1** (Scheme 1). UV/Vis spectroscopy of the aptamer-modified silica nanoparticles confirmed the surface modification with the aptamers and allowed their surface coverage to be calculated. Specifically, from the absorbance at 260 nm, the known extinction coefficient and 2.07 g cm^{-3} density of the silica spheres we estimated the surface coverage with **A1** to be 0.5 molecules nm^{-2}.

Initial studies of the small molecule responsive glass nanopore were performed using the 32-base **A1** aptamer immobilized on the surface of the nanopore. First, the surface of the glass nanopores was aminated using $EtO(Me)_2Si(CH_2)_3NH_2$.[9] Next, we attached the maleimide linker to the surface by soaking the amine-modified nanopores in a DMF solution containing DMAP and SMCC. The electrodes were then treated with the thiol-terminated **A1** which had been reduced using tris-(2-carboxyethyl)phosphine hydrochloride (TCEP). After the aptamer addition, the surface of the nanopore still exposed was passivated with mercaptohexanol to create an aptamer/mercaptohexanol monolayer on the surface of the nanopore. The limiting current of a redox mediator, ferrocene dimethanol ($Fc(CH_2OH)_2$), was measured for the glass nanopore electrodes after each modification, and compared to the initial limiting current (Fig. 3). The limiting current decreased after each modification, confirming that an organic layer has

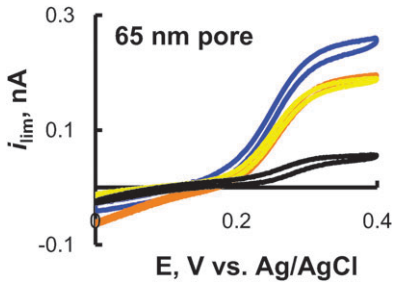


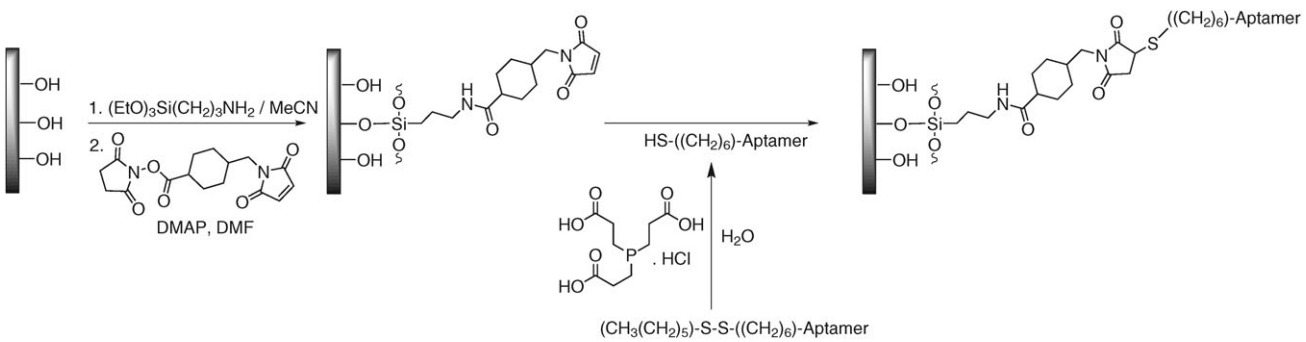
Fig. 3 Representative voltammetric responses of a single nanopore platinum electrode in ($Fc(CH_2OH)_2$) unmodified (blue), amine-modified (orange), maleimide-modified (yellow) and **A1**-aptamer-modified (black). Nanopore orifice radius is shown on the figure.

been formed inside the nanopores, reducing the size of the nanopore orifice and thus reducing the flux of ferrocene dimethanol.

We investigated the response of the **A1**-modified single nanopore electrodes to the addition of the small molecule by employing cyclic voltammetry to monitor the diffusion of $Fc(CH_2OH)_2$ in the presence and absence of cocaine. We employed ferrocene dimethanol, a small, neutral molecule as our redox mediator in order to exclude the possibility that the observed changes in the molecular transport would result from electrostatic effects arising due to the high charge density of the aptamers.[14,15] As expected, the voltammetric limiting current through the **A1**-modified nanopores responds to cocaine: nanopore electrodes of both 20 and 65 nm diameter exhibited increased $Fe(CH_2OH)_2$ transport upon cocaine addition, with the 20 nm nanopores producing a relatively greater change than the larger, 65 nm nanopores (Fig. 4). Removal of the cocaine by washing in potassium phosphate buffer recovered the original current, allowing the nanopore to be reused multiple times (Fig. S2, ESI†).

The cocaine-modulated gating of the glass nanopores presumably arises due to a conformational change in the aptamer where it is converted from partially unfolded conformation with only one of the three junctions formed to a conformation with a fully formed three-way junction containing the cocaine molecule in the internal cavity (Fig. 2). With the formation of the three-way junction, the space that the aptamer occupies inside the nanopores is reduced, allowing for increased transport.

Although analytes other than cocaine were not tested in our work, previous studies have shown[16] that their presence does not affect the folding of the **A1** aptamer immobilized on a solid support in the presence of cocaine. In order to verify that the



Scheme 1 Modification of the glass nanopore surface with aptamers.

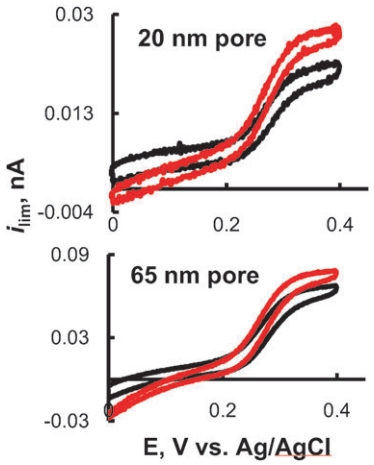


Fig. 4 Representative voltammetric responses of the 32-base **A1** aptamer-modified single nanopore on a platinum electrode in $Fc(CH_2OH)_2$ (black) and in $Fc(CH_2OH)_2$ with 530 μM cocaine (red). Nanopore orifice radii are shown on the figures.

responsive behavior described above results from a change in the conformation of the cocaine-sensing aptamer, the limiting current for a bare single nanopore electrode was examined for pure redox solution and in the presence of cocaine. This bare nanopore electrode did not exhibit any measurable response to cocaine.

In order to improve the gating efficiency we also immobilized two longer aptamer constructs (**A2**, **A3**) on single nanopore electrodes with 65 nm orifices. The gating efficiencies of these longer constructs were approximately twice that of the shorter parent aptamer when employed in nanopores of the same size (Table S1, ESI†), suggesting a more efficient blocking of the nanopores by the larger biomacromolecules.

In order to gain insight into the gating mechanism of the aptamer-modified nanopores, we measured the concentration response for single nanopore electrodes with orifice radii of 65 nm modified with aptamers **A2** and **A3** (Fig. S3, ESI†). The **A2**-modified nanopore electrodes produce near saturated gating at *ca.* 150 μM cocaine, which is just below the ~165 μM dissociation constant reported for this aptamer.[16–18] The **A3**-modified nanopore electrodes produce near saturated gating at *ca.* 230 μM cocaine, similar to the reported dissociation constant (~230 μM) for this aptamer.[16–18] Below these concentration, no gating was observed for either aptamer. This observation and the somewhat non-hyperbolic concentration-response curves (Fig. S3, ESI†) are consistent with the suggestion that a large fraction of the aptamers must be bound and folded before a significant change in transport is observed.

We also measured the response for single nanopore electrodes with orifice radii of both 20 nm and 65 nm modified with aptamer **A1** as a function of cocaine concentration and found near saturated gating at *ca.* 235 μM cocaine, which is significantly higher than the dissociation constant of *ca.* 90 μM reported for this aptamer.[16–18] This may be the result of the smaller size of the **A1** aptamer leading to less efficient gating.

In summary, we have shown that single glass nanopores surface-modified with aptamers that fold in response to cocaine binding are a ready means of producing cocaine-responsive nanopores. Specifically, the binding of cocaine to each of three aptamers used to modify our nanopores triggers a large-scale change in the conformation of the aptamer that, in turn, allows the orifice of the nanopore to be reversibly switched from a more blocked to a more opened state. Consistent with the proposed mechanism, the observed gating efficiency is a strong function of the relative size of the gating aptamer and the orifice diameter. These findings demonstrate the possibility of creating biomimetic nanopores with molecular gating driven by a specific recognition of a small molecule by a biomacromolecule.

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