



Sodium-sensitive contact lens for diagnostics of ocular pathologies

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ABSTRACT

The ability to measure all the electrolyte concentrations in tears would be valuable in ophthalmology for research and diagnosis of dry eye disease (DED) and other ocular pathologies. However, tear samples are difficult to collect and analyze because the total volume is small and the chemical composition changes rapidly. Measurements of electrolytes in tears is challenging because typical clinical assays for proteins and other biomarkers cannot be used to detect ion concentrations tears. Here, we report the contact lens which is sensitive to sodium ion (Na^+), one of the dominant electrolytes in tears. The Na^+ ions in tears is diagnostic for DED. Three sodium-sensitive fluorophores (SG-C16, SG-LPE and SG-PL) were synthesized by derivatizing the sodium green with 1-hexadecyl amine, 1-oleoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine or poly-L-lysine, respectively. These probes were bound to modern silicone hydrogel (SiHG) contact lens, Biofinity from Cooper Vision. Doped lenses were tested for sodium ion dependent spectral properties of probes within the contact lens. The probes displayed changes in intensity and lifetime in response to Na^+ concentration, were completely reversible, no significant probe wash-out from the lenses, were not affected by proteins in tears and were not removed after repeated washing. These results are the first step to our long-term goal, which is a lens sensitive to all the electrolytes in tears. We presented design, synthesis and implementation of three new sodium sensitive probes within a silicon hydrogel lens. Contact lenses to measure the other electrolytes in tears can be developed using the same approach by synthesis and testing of new ion-sensitive fluorophores.

1. Introduction

Modern medical practice depends on diagnostic testing of blood samples. Measurements of electrolytes and some protein concentrations in blood are routinely used to determine the overall health status of patients. Vast array of more specialized tests are used to measure other target analytes such as cholesterol, lipoproteins, hormones and cancer biomarkers [1–5]. These tests are informative because the blood samples are readily obtainable without perturbation of the concentrations of the species of interest.

In contrast to other medical specialties, far fewer tests are available in ophthalmology, and many diagnoses are based on visual examinations [6,7]. This difference is due to the difficulties in obtaining tear samples which are not perturbed by the sample collection process. Tear fluid is produced by at least three processes. Basal level tears keep the

cornea continually wet, lubricate the surface and clean out dirt and other particles. Reflex tears are produced when the eyes are touched or irritated by foreign particles or vapors, such as from onions or pepper spray. Psychic tears are produced during crying and are induced by emotional stress or physical pain [8]. Each types of tears have different concentrations of electrolytes, proteins and lipids. It is difficult to obtain samples of basal tears because contact with the eye results in rapid production of additional tear fluids from the other processes which are different from basal level tears [9]. The volume of tears in each eye is about 6 μL [10,11] and it would be very difficult to measure the individual ion concentrations in such a small sample without the use of a complex and expensive method such as isotope dilution mass spectrometry [12,13].

The present status of testing tear sample for DED is limited to either measurement of the rate of tear production or the total electrolyte

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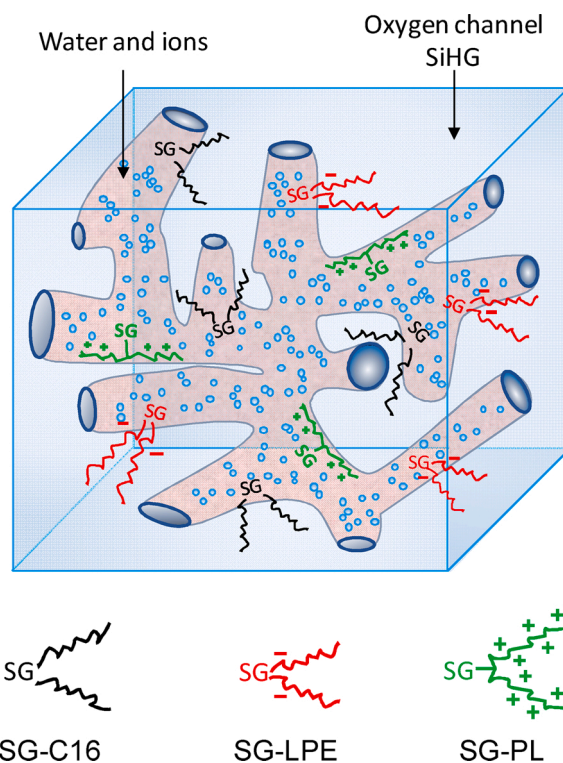
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concentration. Until recently the Schirmer test [14,15] has been the most widely used to estimate the rate of tear production and/or to diagnose DED. The Schirmer test (Scheme 1A) uses pieces of filter paper placed in both eyes and left in place for 5 min. The distance of wetting of the filter paper represents the rate of tear production. Diagnosis based on the wetting distance is somewhat subjective. The Schirmer test is now being supplemented by in-office instrumental measurements of the total electrolyte concentration, which is reported as the total tear milli-osmolality (TmOsm). A presumed unperturbed sample of basal tear fluid is obtained by momentary contact with a testing device shown in Scheme 1B, which measures the electrical conductivity of the sample [16–18] and calculates the total mOsm. The TmOsm is presently regarded as a reliable diagnostic test for DED [19–22]. However, the total mOsm does not provide the concentrations of the individual ions in tears, which limits the usefulness to reveal the form of DED present for an individual patient, or the diagnosis of other conditions. DED affects over 20 million people in the USA, and the prevalence is likely to increase in the aging population [23–25]. The effects of DED are not trivial and have a significant effect on the quality of life, workplace productivity and social interactions. DED can make it difficult for individuals to read printed material or electronic displays, resulting in decreased productivity and quality of life, workplace productivity and social interactions.

Our long-term goal is to develop a contact lens which provides measurements of each electrolyte in tears, without perturbation of the tear composition (Scheme 1C and D). This goal is now feasible because of changes in the polymer chemistry used for contact lenses. The new generation of lenses based on silicone hydrogel (SiHG) contain non-polar regions of silicone and polar regions containing water or tear fluid. These SiHG lenses contain networks of silicone or water which extend across the entire lens. These are referred to as an interpenetrating polymer network (IPN). The interface region of the IPN provides a location to bind ion-specific fluorophores which remain in the aqueous channels in contact with the electrolytes (Scheme 2). These ion-specific fluorophores will contain non-polar groups which bind to the silicone-rich regions. The feasibility of this approach was demonstrated in recent papers using pH, chloride or glucose-sensitive fluorophores with hydrophobic side chains which bind to the silicone-rich regions of the lenses [26,27]. In the present paper we extended this concept using the sodium-sensitive fluorophore Sodium Green [28] by attachment of two different hydrophobic side chains. Contact lens polymer chemistry continues to evolve and some lenses contain only a small amount of silicone. To accommodate these changes, we also synthesized a sodium-sensitive fluorophore linked to poly-L-lysine (PL) for binding at the interface or electrostatic binding to the negative charges in SiHG or non-silicone hydrogel (HG) lenses [29–31]. In the present report these concepts are validated by the testing of three sodium-sensitive



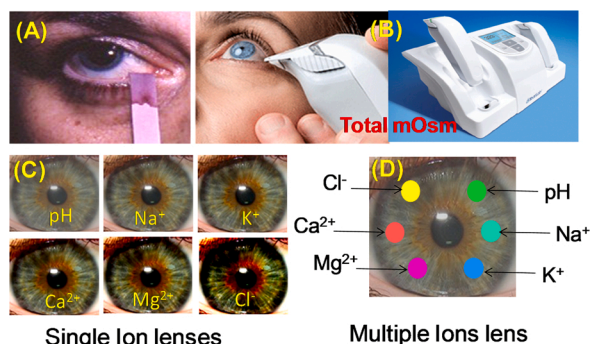
Scheme 2. Schematic representation of interpenetrating polymer network in a contact lens and potential binding sites for the sodium green probes.

fluorophores which bind to the SiHG (Comfilcon A, Biofinity®) lenses and result in a sodium-sensitive contact lenses (Scheme 1C).

2. Materials and methods

2.1. Synthesis of sodium-sensitive fluorophores

We synthesized three sodium-sensitive fluorophores which are derivatives of Sodium Green (SG) (Fig. 1). SG-C16 was prepared using NHS + EDC-activated amide bond formation reaction with two carboxyl groups of SG with two 1-hexadecylamines. A second hydrophobic sodium probe was prepared using the same method to link SG with two molecules of 1-oleoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine (18:1 Lyso-PE, Avanti Polar Lipids Inc), forming SG-LPE. The third sodium probe was SG linked to poly-L-lysine (Sigma-Aldrich, MW = 70–150 kDa). In a typical reaction, to a solution of SG (tetramethylammonium) salt, cell impermeant, Thermo Fisher Scientific, 1 mg, 6.0×10^{-4} mmol, in 2 mL dimethylformamide (DMF) was mixed with *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC, 0.34 mg, 2.0×10^{-3} mmol) and *N*-hydroxysuccinimide (NHS, 0.2 mg, 2.0×10^{-3} mmol) and stirred overnight at room temperature under an inert atmosphere. To the reaction mixture, either 1-hexadecylamine (0.17 mg, 7.2×10^{-4} mmol) or Lyso PE (0.38 mg, 7.2×10^{-4} mmol) was added and the reaction was continued for additional 6 h. For SG-PL we used 0.15 mL of 0.01 % poly-L-lysine, instead of hydrophobic amines, in water and continued reaction for additional 6 h. As prepared aliquots of these reaction mixtures (0.015 mL) were diluted in 2 mL of water to bind the probes into the contact lens. The advantage of washing unbound reactants, including un-reacted SG from the contact lens alleviates the probe purification step before using them for further study. The probe concentration in doping solution was about 1 μ M. The doped lenses were extensively washed by soaking in adequate amount of buffer solution for few days to eliminate any loosely bound probe from the lenses before being used for the ion responsive studies. Unless stated otherwise, all experiments were performed in 20 mM MOPS buffer, 8 mM KCl, pH 7.3,



Scheme 1. Schematic of current measurements of total osmolality in tears: (A) Schirmer test using filter paper, (B) a total osmolality instrument based on measurements of electrical conductivity (C). Possible electrolyte specific contact lenses for measurement of single ions or (D) multiple ions concentrations in tears for specific ocular diagnostic tests.

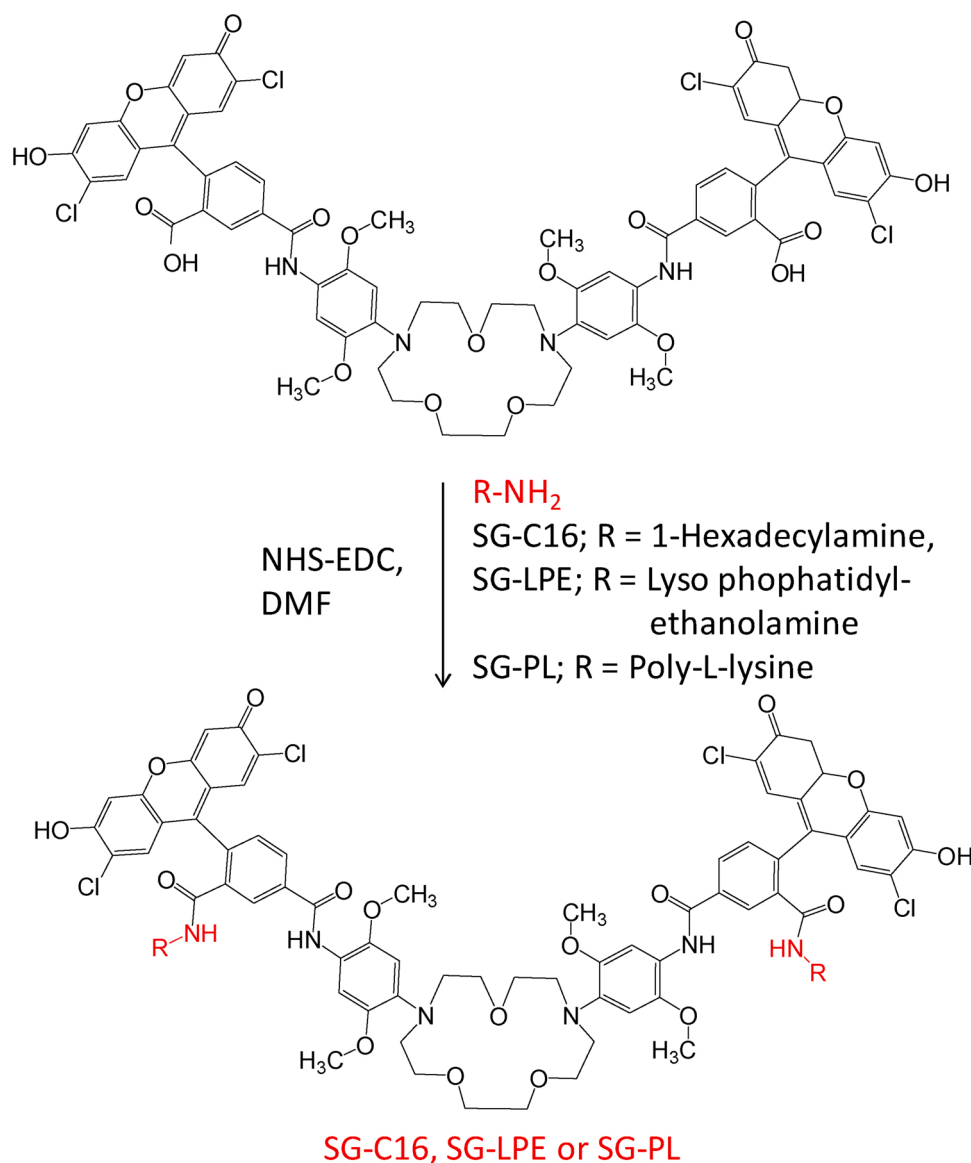


Fig. 1. Synthesis and structures of the three sodium probes for use in contact lenses.

at room temperature, with variable concentrations of NaCl. The emission spectra and lifetimes data for each sodium concentrations were result of five separate experiments.

2.2. Fluorescence spectroscopic measurements

Fluorescence intensities and intensity decays were measured using a FluoTime 300 instrument from Pico Quant (Berlin, Germany) and analyzed with the EasyTau software. The excitation source was a 473 nm laser diode with a repetition rate of 40 MHz and a pulse width of less than 100 ps. Intensity decays were measured at the emission maximum, analyzed in terms of the multi-exponential model

$$I(t) = \sum \alpha_i \exp(-t/\tau_i) \quad (1)$$

where τ_i are the individual decay times and α_i the initial time-zero amplitudes. The mean decay time can be represented in two ways [32]. The average lifetime is given by

$$\tau_f = \frac{\sum \alpha_i \tau_i^2}{\sum \alpha_i \tau_i} = \sum f_i \tau_i \quad (2)$$

where $\sum f_i = 1.0$. The term $f_i = \alpha_i \tau_i$ is proportional to the contribution of

each component to the total intensity decay, and the term $\alpha_i \tau_i / \sum \alpha_i \tau_i$ is the fractional contribution to the steady state emission. In the present report we use the amplitude-weighted lifetime given by

$$\tau_a = \frac{\sum \alpha_i \tau_i}{\sum \alpha_i} \quad (3)$$

which is appropriate when the emission is from a single fluorophore with a changing quantum yield. For a single exponential decay the lifetime is given by $\tau = 1.0/(k_{nr} + \Gamma)$ and the quantum yield is given by $Q = \Gamma/(k_{nr} + \Gamma)$ where k_{nr} is the sum of all non-radiative decay rates and Γ is the radiative decay rate. The value of Γ is determined by the molecular extinction coefficient which remains constant under most conditions [32]. For a single type of fluorophore with a constant value of Γ the normalized α_i values represent the molecular fraction of each decay time component. The value of α_i and τ_i were determined by non-linear least squares fitting using PicoQuant software. Three decay time components were required for the intensity decays. Fluorescence anisotropy decays were determined by individual measurements of the polarized intensity decays with corrections for the G-factor [32].

2.3. Fluorescence microscopy measurements

Fluorescence microscopy and fluorescence lifetime imaging microscopy (FLIM) were performed using a laser scanning confocal microscope from ISS (Champaign, IL) with dual scanning capability. The stage scanner was used to image entire contact lens using image size $1\text{ cm} \times 1\text{ cm}$ using resolution of 128×128 pixels. The galvo scanning mirror was used to image small area with image size of $250\text{ }\mu\text{m} \times 250\text{ }\mu\text{m}$ and resolution of 256×256 pixels. FLIM images were analyzed pixel by pixel using two-component model and average lifetime images displayed as calculated according Eq. 3. The excitation source was a 473 nm pulsed laser diode, with observation at 560 nm (50 nm bandwidth) and a 20X objective.

2.4. Sodium binding affinity

The sodium affinity of the labeled lenses can be described in terms of the dissociation constant (K_D). The dissociation reaction for a probe complexed with Na^+ can be written as



where P is the free probe concentration with no bound sodium, $P\text{-Na}^+$ is the concentration of Na^+ -bound probe, and $[\text{Na}^+]$ is the concentration of free sodium. The dissociation constant K_D is given by

$$K_D = \frac{[P][\text{Na}^+]}{[P - \text{Na}^+]} \quad (5)$$

The ratio of sodium-free and sodium-bound probe is given by

$$\frac{[P]}{[P - \text{Na}^+]} = \frac{K_D}{[\text{Na}^+]} \quad (6)$$

We describe the binding affinities as the sodium concentration at the mid-point of the spectral responses, which is the apparent dissociation constant of the probe-sodium complex. If the probe intensity changes with sodium binding the actual binding constant will be different but will not affect the validity of the measurements.

3. Results

3.1. Selection of probe structure

The structures of the sodium sensitive fluorophores, SG-C16, SG-LPE and SG-PL, were selected by consideration of the composition of SiHG polymers. The structure of SG-C16 contains two non-polar alkyl chains which we expected would bind at the water-silicone interface (Scheme 2). Lyso-phosphatidyl ethanolamine was used in SG-LPE because it is a natural biomolecule, and amphipathic lipids are sometimes used to increase the wettability of the hydrophobic silicone regions [33–35]. Lysolipids differ from typical phospholipids by the loss of one alkyl chain. As a result, lysolipids form micelles which are 2–4 nm in diameter [36–39]. The pore size of a SiHG lens is thought to be around 50 nm [40,41] and we reasoned that smaller micelles would diffuse easily into the water channels and facilitate binding of the hydrophobic sodium probes. SG-PL contains poly-L-lysine (Fig. 1), which is widely used to decrease the hydrophobicity of glass, PDMS and silicone [42,43], which was expected to bind at the interface region of the lenses. It was also possible that polylysine could bind the non-polar regions of SiHG. The monomers used in making SiHG polymers include regions with partially oxidized carbon atoms, carboxyl groups and cross-linkers. These groups can contribute a negative charge to the lens [44,45] and electrostatic binding of polylysine, which could be second generic approach to binding ion-sensitive fluorophore (ISF) to contact lenses.

3.2. Labeling of biofinity lenses with the sodium probes

The lenses were labeled by incubation in 2 mL of a 1 μM solution of the probe. All three sodium probes were found to bind strongly to the Biofinity lenses resulting in uniform emission across the lens which was visible in a darkened room (Fig. 2, top row, left). The rates of probe uptake into the lenses were not studied in detail but SG-PL appeared to bind most rapidly. After an hour the lenses with the three probes were roughly equally intense. We did not determine the amounts of probe bound to the lenses or the amount of probe remaining in the labeling solution. After labeling the lenses were washed several times in 20 mL of probe-free buffer, which is a about 1300-fold larger volume than the volume of a contact lens, which is approximately 15 μL . This washing resulted in about half of the intensity being removed from the lenses. This result is consistent with unbound probe in the aqueous IPN channels being removed by washing. After this initial decrease in intensity the lenses retained the same brightness for a period of few weeks when stored in 3 mL of probe-free buffer (Figure S1).

3.3. Binding of the sodium probes to contact lenses

Sodium probe binding to the lenses was tested by measurements of the anisotropy decays. For the free SG probe the anisotropy decayed quickly to zero (Figure S2). The three sodium probes in lenses displayed a rapid initial anisotropy decay followed by a long correlation time which did not decay to zero during the 1–3 ns lifetime of the fluorophores. The anisotropy decays are similar to those observed for numerous fluorophores bound to large proteins or membranes [46,47]. These results (Figure S2) are consistent with the ion-binding region of the probes being free to rotate in the aqueous channels, but the overall global rotation of the probes limited by the interfacial regions of the lenses.

Strong and essentially complete binding of SG-C16 to the Comfilcon A lenses was confirmed by the confocal fluorescence intensity images (Fig. 2, lower panels). The lens was immersed in the buffer within a small Petri dish and images were acquired with stage scanner at focal planes that allow imaging of entire lens. The intensity outside the lens (from the buffer) was less than 1% from that of the labeled lens. Lifetime analyses were performed on pixels with intensity thresholds above 100 counts. The confocal images appear different, with no intensity in the center, because they were measured at different focal planes. It is important to notice that the FLIM images show essentially identical lifetimes in all regions of the lens. The lifetime changed uniformly from 1.37 ns in the absence of sodium to 3.05 ns in the presence of sodium, which indicates that any region of the lens can be used as lifetime-based sensing for sodium. Similar results were obtained for SG-LPE (Figure S3) and SG-PL (Figure S4), which also show complete binding of the probes to the lenses and increases in intensity and lifetime in the presence of sodium. These results demonstrate that all three sodium probes bound strongly to SiHG contact lenses and respond to sodium concentration.

3.4. Sodium concentration measurements using the labeled contact Lens

The selection of SG for detection of sodium ion in tears is not an obvious choice. This probe is based on an di-azacrown-5, a crown ether with two nitrogen atoms [28]. This parent structure SG was designed to obtain a high affinity for sodium with a binding constant (mid-point) near 6–10 mM [48], which is the physiological range for intracellular sodium. However, the sodium concentration in tears is near 120 mM and comparable to the concentration in blood. We reasoned the sodium affinity of SG bound to contact lenses could be decreased for two reasons. First, the azacrown ether and attached fluorophores could be partially buried in silicone regions of the lenses and less accessible to the aqueous phase. Secondly, the parent fluorophore SG contain two negatively charged carboxyl groups which may contribute to the sodium binding affinity. This role of carboxyl groups for increased sodium affinity of SG

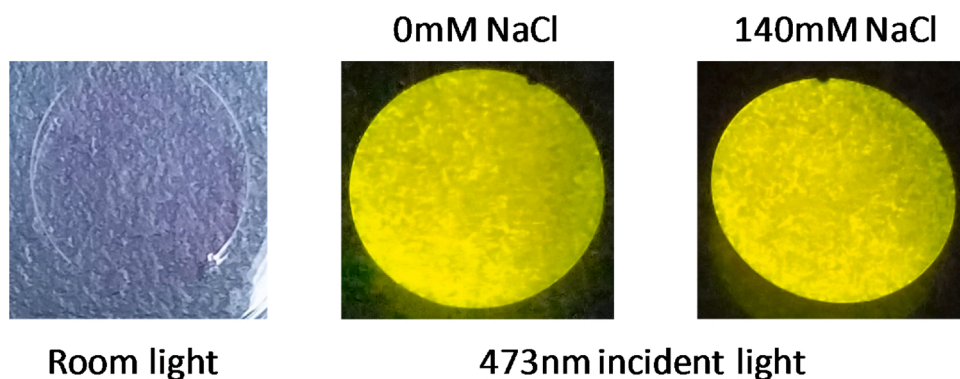
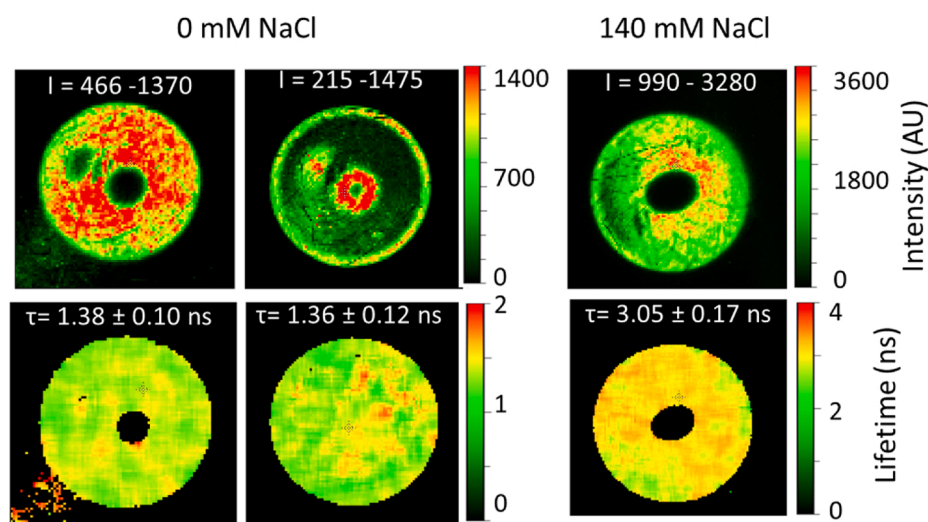


Fig. 2. Top row, photographs of SG-PL in a Biofinity contact lens in room light (left) and with diffused 473 nm laser illumination with 0 and 140 mM NaCl. Bottom rows, confocal intensity and lifetime images of SG-C16 in Biofinity contact lenses for 0 and 140 mM NaCl. The images were acquired with stage scanner with image size of 1cm $\times$ 1cm area and resolution of 128 $\times$ 128 pixels. Images at 0 mM NaCl were acquired at two different focal planes to illustrate that lifetime is not dependent on intensity. Lifetime images were obtained by fitting pixel by pixel to two-component model. Numerical values are average values (Eq. 3) over entire image and standard deviation.



is supported by the weaker sodium binding of CoroNa Green, CoroNa Red and Asanti NaTrium Green [49], which do not have negative carboxyl groups near the sodium binding sites. Negatively charged carboxyl groups are not present in the three sodium probes (Fig. 1) used in the present study.

Fig. 3 shows the effects of different sodium concentrations on the fluorescence intensities and lifetimes of SG-C16 in Biofinity lens. The fluorescence intensities increased about 3-fold as the sodium concentration is increased from 0 to 150 mM. The emission spectra do not change shape and the emission maxima remains the same, which would make it difficult to use the intensities alone to determine tear sodium concentrations. Addition of a reference fluorophore, which is not sensitive to sodium, would allow wavelength-ratiometric sensing using SG-C16 [50,51].

An alternative approach is to use the intensity decays or lifetimes to measure sodium concentrations. Importantly, the intensity decay rate of SG-C16 decreases (Fig. 3) and the fluorescence lifetime increases at increased sodium concentrations. This allows lifetime-based sensing of sodium. Detailed lifetime analysis for SG-C16 is shown in Table S1. Similar changes in intensity and lifetime were observed for SG-LPE and SG-PL (Figures S5 and S6).

Intensity- and lifetime-based sodium titration curves for parent fluorophore SG in buffer and the three probes in lenses are shown in Fig. 4. The multi-exponential analyses are summarized in Tables S1-S4. SG in buffer displayed a binding affinity near 6.5 mM, which is too high for measurements of sodium in tears (Figs. 4 and S6). The sodium affinity was decreased to values near 30 mM for the three probes bound to Biofinity lenses. Changes in intensity and lifetime continue through the

physiological sodium range in tears of 120 mM, but the changes are smaller above 150 mM sodium. We expect the sodium probes for the final electrolyte contact lens (EL-CL) will require slightly weaker sodium binding. This can be accomplished by modification of the sodium binding structure by the use of crown ethers with no nitrogen atoms or mono-azo crown ethers [52,53]. Additionally, the sodium affinity may be dependent on the type of contact lens polymer. The essential point is that sodium concentrations can be determined with labeled contact lenses and the sodium binding affinities can be adjusted to match the physiological sodium concentration in tears.

3.5. Reversibility and effects of potential interfering proteins

A clinically useful sodium contact lens must display a reversible response to sodium. The sodium-dependent lifetime and intensity changes for SG-C16 were completely reversible for concentrations changes from 0 to 220 mM NaCl (Fig. 5). The lenses were washed several times in 20 mL of probe-free buffer for each cycle to remove sodium and/or any unbound probe. The constant fluorescence intensities demonstrate that SG-C16 is not washed out of the lenses. Similar reversibility and absence of probe washout were observed for SG-LPE and SG-PL (Figures S8 and S9).

Tears contain a large number of other electrolytes (including potassium, calcium, and magnesium ions) and proteins like lysozyme, mucin, human serum albumin and other water-soluble glycoproteins which could affect the sodium response. Sodium Green selectivity to sodium ion was previously established [48]. Monovalent alkali ions show size-dependent binding with crown ethers and aza-crown ethers.

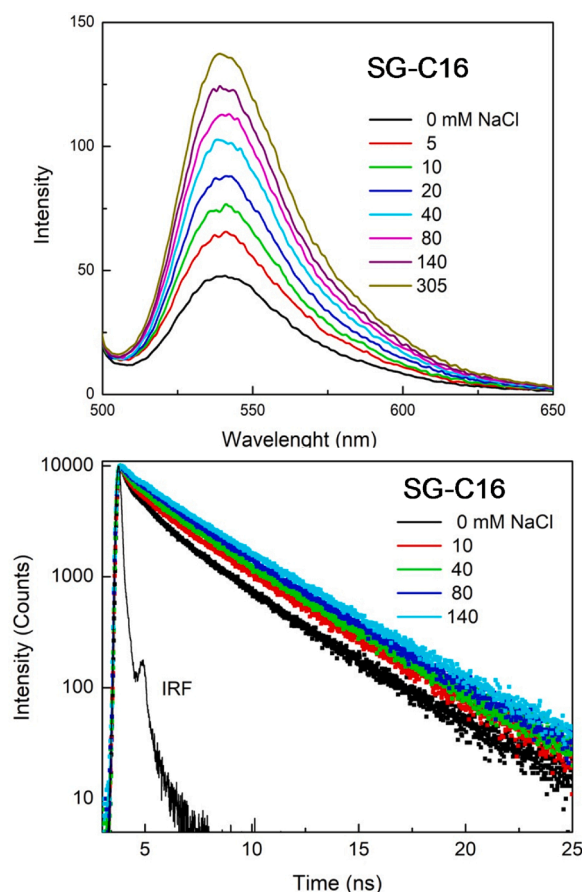


Fig. 3. Sodium-dependent emission spectra (top) and intensity decays of SG-C16 in Biofinity contact lens.

Diaz-crown-5 moiety of sodium green exhibit 41-fold selectivity to Na ion over K ion and no significant binding interactions were noticed with divalent Ca^{2+} and Mg^{2+} . Considering this we tested SG-PL sodium response in the presence of excessive potassium ion concentration (Fig. 6), which shows the insignificant interference of K^+ ion on Na^+ response of the lens. The mid-points for SG-PL sodium response in the presence of potential interferents are presented in Fig. 6. It was not practical to test all protein components in tears. We selected three proteins. Lysozyme was selected because it is the most abundant and comprises 20–40 % of tear proteins [54]. Lysozyme binds quickly to many contact lenses which is thought due to its net positive charge at neutral pH and the negative charges on most lenses. Serum albumin is present in tears at low concentrations but can increase under some conditions [55]. Serum albumin is known to bind hydrophobic molecules [56] so binding to silicone was a possibility. We also tested mucin type II (MUC2) which is 80 % oligosaccharide by weight and is present in tears in a freely diffusing form [57]. The sodium responses of SG-PL were completely unchanged in the presence of 1 mg/mL of all three proteins (Fig. 6).

4. Discussion

4.1. Contact Lens to measure all electrolytes in tear fluid

In the present report we describe three different sodium-sensitive fluorophores that bind to SiHG contact lenses and display spectral changes over the normal sodium concentrations in tears. All fluorophores showed the response to sodium to be completely reversible, no significant probe wash-out from the lenses, and no interference from three proteins commonly present in tears. These results are the first step

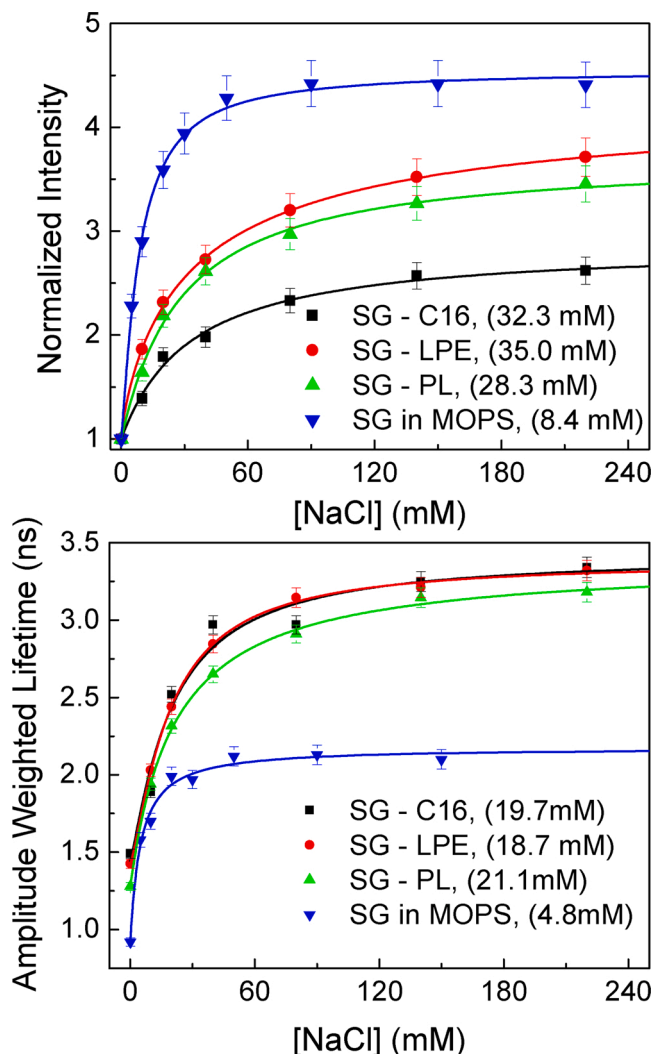


Fig. 4. Sodium-dependent intensities and lifetimes for SG in MOPS buffer and the three sodium probes in Biofinity lenses. Lifetime measurements were performed on FluoTime 300 instrument. Numerical values correspond to mid points of sodium-dependent responses.

to our long-term goal, which is a lens sensitive to all the electrolytes in tears (EL-CL).

A limiting factor to making an EL-CL has been the lack of ion-specific fluorophores which bind to contact lenses and respond to tear electrolytes. There is a vast literature describing ion-sensitive (ISF) fluorophores which are sensitive to the electrolytes such as pH, Na^+ , K^+ , Ca^{2+} , Mg^{2+} and Cl^- [58–61]. However, most of these ISF are designed to be water soluble. As a result, they will be rapidly washed out from contact lens by tear fluid, which is replaced every 5 min [62]. Additionally, most of these probes are designed for use at intracellular ion concentrations, which can be different from the physiological concentrations in tears. The polymer chemistry for contact lenses has changed dramatically and allows design of ISF which bind to the lenses. The IPN structure provides a polar to non-polar interface regions for binding of ISF, and the channels extend from the front to the back of the lens for rapid ion transport. The previous generation of HEMA non-silicone hydrogel-based contact lenses are spatially uniform and do not have such an interface. We have had previous failures in sensing analytes using these non-silicone HEMA-type lenses [63,64]. The present results for sodium provide a roadmap for synthesis of ISF for all tear electrolytes.

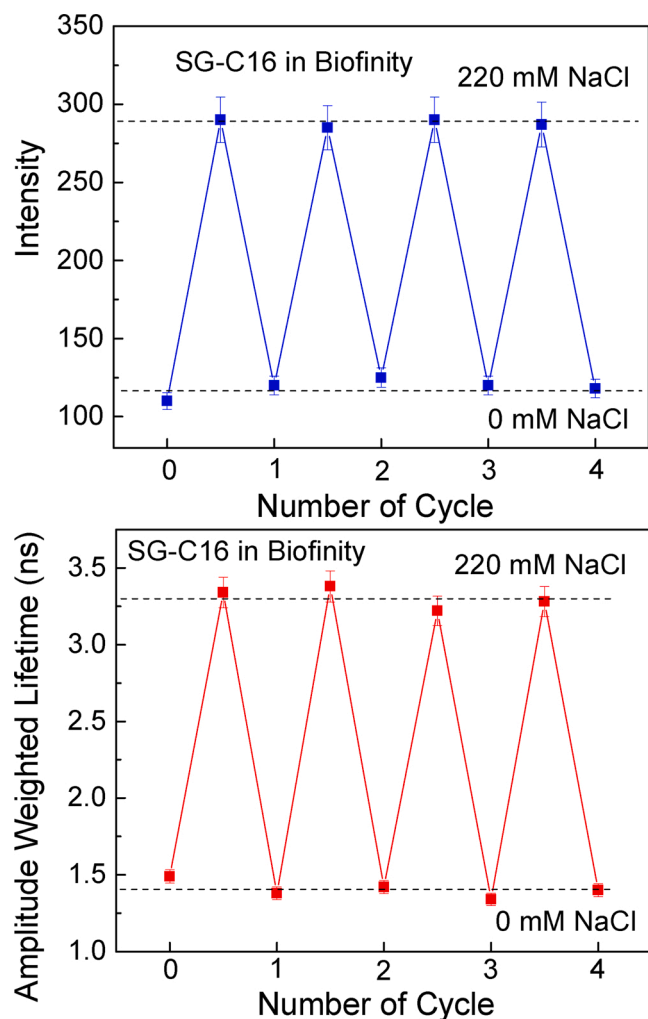


Fig. 5. Reversibility of a SG-C16-labeled Biofinity contact lens measured by intensity (top) and lifetime (bottom) with repeated cycling between 0 and 220 mM NaCl. Measurements were performed on the center area of the lens using FLIM instrumentation described in materials section.

4.2. Limitations of present electrolyte measurements in tears

The Schirmer test (Scheme 1A) is used for diagnosis of dry eye disease (DED). A strip of filter paper placed in the conjunctival sac in patients and the wetting distance is measured. A shorter wetting distance suggests the presence of aqueous deficient dry eye (ADDE). This test is difficult to standardize and there is no accepted wetting distance to diagnose ADDE [65,66]. Additionally, DED is a multi-factional disease which can be due to other causes such as Sjogren's syndrome, which is an autoimmune reaction affecting the moisture secreting glands of the eyes and mouth [67,68]. The total electrolyte concentration in tears can also be due to a change in lipid composition. Such changes can be due to dysfunction of the Meibomian gland resulting in an excessive rate of water evaporation from tears [69,70], resulting in increased electrolyte concentrations. This condition, called evaporative dry eye (EDE), is difficult without a detailed analysis of the lipid composition of tears [71, 72]. It is reasonable to suggest that the difficulties in diagnosis of DED and other disorders are due to limited testing that can be performed using tears.

The most modern measurement of tear electrolytes is obtained from the electrical conductivity of a tear sample. The sample is taken quickly prior to the eye responding to physical contact (Scheme 1B) and presumed to be a basal tear sample [16–18]. The measurement appears to be simple but three (3) repeated measurements on each eye are required

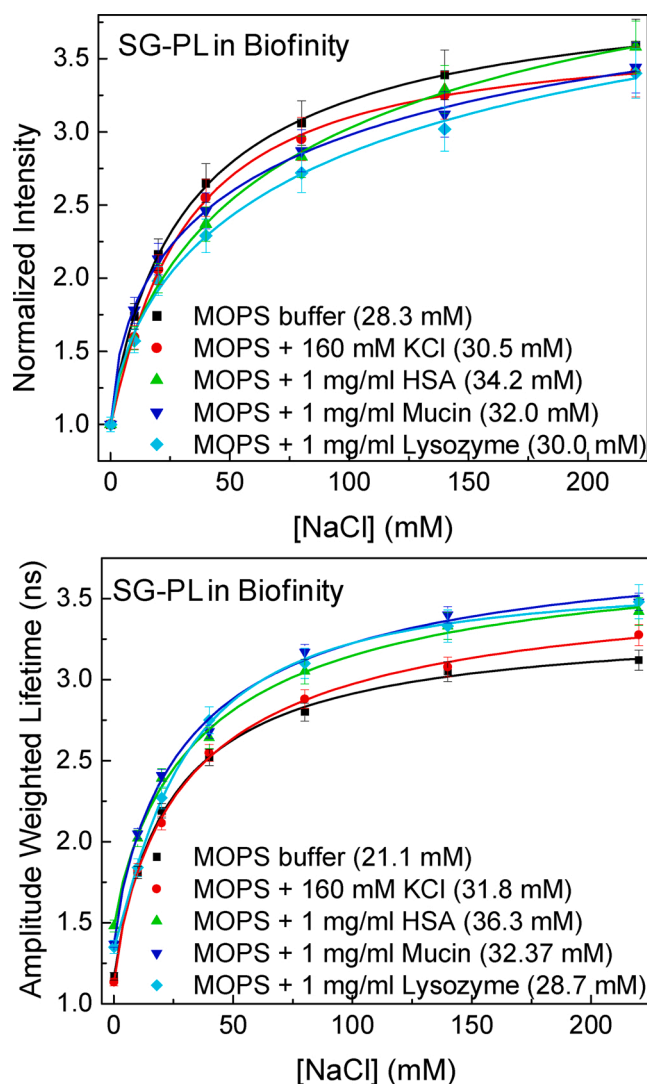


Fig. 6. Sodium-dependent intensity (top) and lifetime responses (bottom) of SG-PL in Biofinity lenses in the absence and presence of the KCl, HSA, mucin or lysozyme. Measurements were performed on the center area of the lens using FLIM instrumentation. Numerical values correspond to mid points of sodium-dependent responses.

for a clinically reliable result [73–75]. There has been a report of a microfluidic system for measurement of Na^+ , K^+ , Ca^{2+} and pH [76]. This device provides a one-time measurement and is still to approved for clinical use.

4.3. Usefulness of measuring individual electrolyte concentrations

Because the individual ion concentrations not presently available it is difficult to provide a clinical justification for measuring each electrolyte. Some publications suggest the ion concentrations in basal tear fluid can be diagnostic for various medical conditions. For example, inflammation of the cornea is known to be associated with neutrophils [77,78]. Activated neutrophils release protons [79–81] and a decrease in pH may indicate an infection. Potassium has been reported to play a role in UV protection [82,83]. Calcium plays a role in mucin packaging [84] and increased calcium concentrations have been linked to cystic fibrosis [85] and age-related macular degeneration [86]. Magnesium deficiency has been linked to the incidence of glaucoma [87–89]. The linkage between various pathologies and individual ion concentrations is likely to be clarified when more separate electrolytes can be measured, without

purification of the basal tear fluid.

4.4. Drug development for DED and other ocular pathogens

It appears that drug development for DED has been hindered by the limited information from measurements of the total electrolyte concentrations. There are only two FDA-approved drugs to treat DED. Ristasis (approved in 2003) is an emulsion of cyclosporine 0.05 %, an immunomodulator, which decreases the inflammatory response in the conjunctival epithelial cells [90,91] and increases tear production. Restasis is expected to be effective for patients with ADDE. The difficulty in ocular drug development is seen from the 14 years before the FDA to approve a second drug Xiidra (Lifitegrast 5%) in July 2016 [92,93]. This drug inhibits T-cell binding to receptors and decreases the release of inflammatory cytokines [94,95]. It is possible that measurement of individual ion concentrations will stimulate research efforts to modify these concentrations.

4.5. Use of an electrolyte contact Lens (EL-CL) in an ophthalmology office

It is easy to imagine how an EL-CL could be used in an ophthalmology office. Contact lenses can be easily inserted even for individuals who do not normally wear contact lenses. After insertion of the lens there can be a short waiting period of about 10 min for the tears to regain the normal electrolyte concentrations. The fluorescent signals could then be used to determine the ion concentrations. The EL-CL also could be a prescribed lens to be worn continually by the patients for self-testing with an EL-CL reading device. Such a device is now possible due to the many advances in electronics, detectors and iris tracking technologies.

4.6. Devices to measure fluorescence from contact lenses

The rapid evolution of consumer electronics indicates it is now possible to design and fabricate an EL-CL reader for intensity ratios or lifetimes from labeled lenses worn by patients. We expect the first device would be located at the eye examination station in the ophthalmologist's office. This device will be a small desktop-type steady-state fluorometer for wavelength-ratiometric measurements and/or a time-resolved instrument for intensity decays. All the electronics for time-correlated single photon counting can be placed on a single computer circuit board. After contact lenses are developed for additional tear electrolytes these instruments will be used for research and diagnosis of DED and other ocular conditions. It seems likely that clinical research projects will be initiated to correlate individual ion concentrations with specific ocular diseases. After demonstration of clinical value we expect smaller EL-CL readers will be developed, such as a portable hand-held device possibly based on a cell phone camera. The following paragraphs describe current optical technologies for such devices.

4.7. Light sources and detectors for fluorescence

Many solid-state LEDs and laser diodes (LD) are available for most wavelengths above 260 nm in the UV and extending to over 900 nm in the NIR [96,97]. Pulsed laser diodes (without frequency doubling) are available down to 375 nm. Wavelengths below 400 nm are absorbed by the cornea and do not reach the retina [98,99]. Shorter wavelengths are blocked by many contact lenses [100,101]. Most ISF absorb and emit light in this spectral range. These fluorophores can be used as starting points for making probes which are functional in SiHG lenses.

4.8. Fluorescence and FLIM images of the EL-CL

The rapidly evolving technology for CMOS detector arrays will enable hand-held and battery powered EL-CL reading devices that can

measure the intensities and lifetimes in different locations on a contact lens (Scheme 1D). Charge-coupled devices (CCDs) are rapidly being replaced by CMOS detector arrays (CDAs) because CDAs require 100-fold less power than CCDs [102,103]. CDAs have high sensitivity and frame rates [104] and have been used for live cell imaging and single molecule detection [105,106]. CDAs are capable of measuring nano-second decay times and therefore useful for fluorescence lifetime imaging microscopy (FLIM) [107,108]. A new CDA was announced which obtained 3D images using the time-of-flight from camera-to-surface and back [109]. Since the distance resolution appears to be below 1 inch the time resolution must be below 1 ns.

4.9. Iris tracking technology

Measurements of multiple ions or multiple spots on a lens may be difficult when the exact iris location is not known and the iris may be moving. Devices are already available for identification of individuals by imaging the iris, so the software and hardware to image a moving iris is already available. The EL-CL will contain multiple locations which provide known emission intensities and/or lifetimes for calibration, and thus be self-calibrating. The availability of point-of-care and self-testing measurements of tear electrolytes will provide an immediate health benefit for individuals with DED. We expect the EL-CL will become widely used for diagnosis and treatment for other ocular pathologies.

And finally, we note that our sensing contact lens is distinct from those being developed elsewhere which contains electronic circuits, and antennas to capture energy to power the device, and detectors to measure the emission [110–113]. The complexity and expense of such electronic contact lenses will increase greatly for measurements of multiple electrolytes. Such lenses are likely to be expensive, not suitable for one day use and available in only a single type of contact lens polymer. Our approach does not require any electronics in the lenses. Addition of probes is likely to be inexpensive and the labeled lenses will be compatible with the growing trend toward one-day use of contact lenses.

5. Conclusion

In summary, we presented sodium-sensitive SiHG contact lenses by placing ion sensitive probes, sodium green derivatives SG-C16, SG-LPE and SG-PL. The probe sodium green was derivatized with hydrophobic or poly-lysine chains to bind the silicone regions of lenses, while maintaining the ability to sense sodium ions in aqueous regions of the lens. The contact lens reversible response to sodium is useful for continuous monitoring. To the best of our knowledge, this is the first report of a fluorescent contact lens sensitive to sodium concentration. Water-soluble probes are already known for other dominant electrolytes in tears. Our present approach can allow rational design of contact lenses for the total electrolyte profile of tears.

Availability of data and materials

The data analyzed during the current study are available from the corresponding author on reasonable request.

CRedit authorship contribution statement

Ramachandram Badugu: Conceptualization, Investigation, Writing - original draft. **Henryk Szmazinski:** Investigation. **E. Albert Reece:** Writing - review & editing. **Bennie H. Jeng:** Writing - review & editing. **Joseph R. Lakowicz:** Conceptualization, Supervision, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

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