ACS Biomaterials SCIENCE & ENGINEERING

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Review

Lifting the Veil: Characteristics, Clinical Significance, and Application of β-2-Microglobulin as Biomarkers and Its Detection with Biosensors

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Cite This: *ACS Biomater. Sci. Eng.* 2022, 8, 3142−3161

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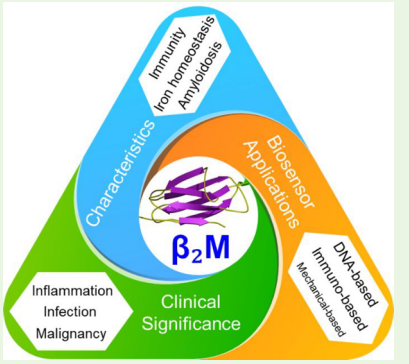
ABSTRACT: Because β-2-microglobulin (β_2M) is a surface protein that is present on most nucleated cells, it plays a key role in the human immune system and the kidney glomeruli to regulate homeostasis. The primary clinical significance of β_2M is in dialysis-related amyloidosis, a complication of end-stage renal disease caused by a gradual accumulation of β_2M in the blood. Therefore, the function of β_2M in kidney-related diseases has been extensively studied to evaluate its glomerular and tubular functions. Because increased β_2M shedding due to rapid cell turnover may indicate other underlying medical conditions, the possibility to use β_2M as a versatile biomarker rose in prominence across multiple disciplines for various applications. Therefore, this work has reviewed the recent use of β_2M to detect various diseases and its progress as a biomarker. While the use of state-of-the-art β_2M detection requires sophisticated tools, high maintenance, and labor cost, this work also has reported the use of biosensor to quantify β_2M over the past decade. It is hoped that a portable and highly efficient β_2M biosensor device will soon be incorporated in point-of-care testing to provide safe, rapid, and reliable test results.



KEYWORDS: *β-2-microglobulin, biomarker, biosensor, major histocompatibility complex, immunoassay, glomerular filtration rate*

1. INTRODUCTION

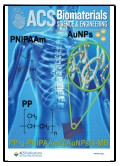
For decades, clinical medicine has relied on the use of biomarkers to indicate various health statuses and disease characteristics.[1] Unfortunately, there is an unmet need for a versatile biomarker that indicates multiple diseases and health threats in patients. In view of this, extensive clinical and validation methods are essential to identify new biomarkers that hold promising outcomes for the future of healthcare.[2] The advancement and maturity of technologies in recent years may enable the search for novel biomarkers to achieve a new milestone in precision medicine. However, the full potential of biomarkers cannot be examined unless significant challenges are first overcome.

This review highlights the potential use of β-2-microglobulin (β_2M) as a versatile biomarker in numerous medical applications. Before delving into an in-depth discussion of biosensing, this work first describes the fundamental biology of β_2M, particularly its significance and impact on clinical medicine. By reviewing literature, a β_2M-based equation with which to assess renal function was formulated. Past data obtained before the practicality of utilizing analytical assays in biomarkers was also discussed. Next, some light was also shed on how β_2M biosensors have been utilized and characterized by extant studies. In the end, this work concluded by highlighting the challenges faced by the current biosensors as well as trends that need to be overcome and how to address them. In brief, this review aims to educate researchers, developers, and partners on the multifaceted role of β_2M, while facilitating them to design and spur the development of β_2M biosensors.

1.1. Characteristic and Functionality.

β-2-Microglobulin is a low molecular weight protein (LMWP; 11800 Da; size, 11 Å) of 99 amino acids that is encoded by a gene in chromosome 15 in humans. Its tertiary structure is a classic β-sandwich that is typical in the immunoglobulin (Ig) domain. However, instead of forming dimers, β_2M constitutes the extracellular light chain of the major histocompatibility complex class I (MHC-I) molecules family.[3] It is universally expressed on the surface of all nucleated cells, particularly immunocompetent cells, such as lymphocytes and monocytes.[4] The importance of β_2M lies in the cell surface expression and structural stability of

Received: January 11, 2022
Accepted: July 7, 2022
Published: July 18, 2022



the MHC-I molecule. A noncovalent interaction between β_2M and the heavy α chain of MHC-I is necessary for the antigen presentation by cytotoxic (CD8+) T lymphocytes. Its influence also extends into the process of inflammation, complement cascade, and stress response.

An additional function of β_2M is iron homeostasis in association with hemochromatosis and the HFE protein. The heterodimeric HFE/β_2M protein regulates the expression of hepcidin messenger ribonucleic acid (RNA) in the liver, and its interaction with transferrin receptor-1 (TfR1) further regulates hepcidin levels in response to circulating amounts of transferrin.[5] The loss-of-function of either protein, as is the case in type I hereditary hemochromatosis, leads to decreased plasma hepcidin levels, increased transferrin saturation, and, subsequently, an overload of iron in tissues.[6−9] This widespread genetic disorder, which occurs as a result of autosomal recessive inheritance, mainly affects Caucasian men and women aged between 40 and 60 years old.

1.2. Molecular Structure, Amyloids, and Variants. Because β2M is a uremic solute, it is a LMWP. These are proteins with a molecular weight of between 12 and 26 kDa that physiologically undergo free glomerular filtration and are almost completely reabsorbed by the intact proximal tubule.[10] However, some studies classify β_2M as a middle molecule according to the new definition that the European Uremic Toxin Work Group has proposed. However, because the new middle molecule category encompasses toxins with molecular weights ranging between 0.5 and 60 kDa, it comprises nearly all LMWPs.[11,12] The Protein Data Bank (PDB) recorded the molecular weight of β_2M as 11.88 kDa, which most studies have agreed with its value.[13−15] Nevertheless, another reported slight deviation as 11.7 kDa.[16]

The molecular structure of wild-type (wt) β_2M, its typical form in nature, consists of seven antiparallel β-strands, labeled A−G. These strands are organized into two large antiparallel β-sheets, comprising strands A-B-E-D and C-F-G, respectively, are schematically shown in Figure 1.[3,17−19] These sheets are linked by a single disulfide bridge (Cys25-Cys80) between cysteine residues at position 25 on the B-strand and position 80 on the F-strand. This cross-linked bridge plays a pivotal role in β_2M fibrillogenesis.

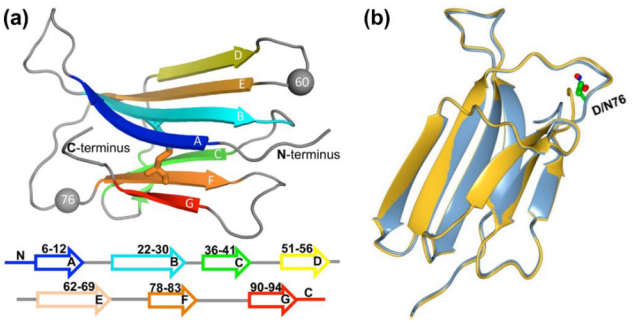


Figure 1. Schematic representation of β-2-microglobulin (β_2M) structure. (a) Three-dimensional structure of β_2M showing the seven β-strands, organized in two β-sheets (comprising strands A-B-E-D and C-F-G, respectively), and the positions of Trp60 and Asp76, which is mutated to Asn in the D76N mutant (PBD code 4FXL). Reproduced with permission from ref 17. Copyright 2020 Loureiro and Faisca under Creative Commons CC BY 4.0 license, published by Frontiers. (b) Structural analysis of the D76N mutant (blue) compared to that of the wild-type protein (yellow) shows that two proteins are perfectly superimposable (PBD code 2YXF). Reproduced with permission from ref 19. Copyright 2019 The Authors under Creative Commons CC BY 4.0 license, published by Multidisciplinary Digital Publishing Institute.

In the event of protein misfolding, amyloidosis occurs, where the state solubility of the native protein aggregates into insoluble and highly organized amyloid fibrils.[20] Best known as dialysis-related amyloidosis (DRA) in β_2M cases, these conditions are often associated with various pathological conditions. The blood β_2M levels of patients undergoing long-term (>10 years) hemodialysis (HD) for end-stage renal disease (ESRD) is 50−60 times higher than that of healthy individuals which could predispose them to DRA.[21,22] Despite recent technological advances in dialysis, the rate of β_2M production still exceeds the rate of β_2M clearance as the cutoff molecular weight of the dialysis membrane is similar to or lower than the molecular weight of β_2M.[17,18,23] Because a standard HD session partially filters out β_2M, several dialysis techniques, such as the adaptation of high-flux membranes, increased HD duration, and hemodiafiltration, have been suggested to improve β_2M clearance.[24] The direct orthopedics complications of DRA are closely related to the discovery of the end-products of advanced glycation in β_2M amyloids formed glycosylated β_2M molecules.[25]

Over the years, multiple studies have extensively investigated β_2M aggregation. β_2M amyloids may be found in naturally occurring variants (e.g., ΔN6, ΔLys58, and Asp76Asn) or in engineered mutant variants (e.g., Asp59Pro, Trp60Cys, Trp60Val, and Trp60Gly).[17] The occurrence of specific loop mutations has been found to be the primary determinants of the thermodynamic stability and aggregation propensity of β_2M variants.[17,19] Unlike wt β_2M, these variants have either low kinetic or thermal stability, which makes it easier for them to aggregate and form amyloids. The Asp76Asn variant has been identified as the cause of rare types of hereditary systemic amyloidosis. It is noteworthy that the disease occurs in the absence of chronic kidney disease and with the detection of serum β_2M.[26]

1.3. Physiological Effect of Changes in β_2M Levels. The highest concentration of serum β_2M is detected at birth, gradually declines within the first two years of life, and stabilizes thereafter. The amount of urinary β_2M is higher in premature infants and decreases as the gestational age increases, irrespective of birth weight or weight loss in the first week of life.[27,28] The amount of urinary β_2M detected during infancy is higher than that of adults (0.65 mg L^{-1}) and differs between genders. This is because the kidneys continue to grow and only reach full functional maturity in early infancy, as the basolateral tubular Na−K-ATPase pump is still low.[28−30] The immaturity of the nephrons, particularly in the proximal tubules, is evidenced by decreased reabsorption and increased β_2M excretion.

Under normal physiological conditions, β_2M is continuously secreted at a low level due to membrane turnover into various body fluids, most notably in synovial serum and urine.[31,32] Serum β_2M concentrations usually range between 1.5 and 3 mg L^{-1} and are roughly 25% lower in cerebrospinal fluid (CSF),[33] while lacrimal fluid (human tears) contains 0−20 mg L^{-1} β_2M.[34] The kidneys are primarily responsible for β_2M elimination. Although glomeruli filter out relatively smaller sized β_2M, it is mostly reabsorbed and catabolized to amino acids at the proximal convoluted tubules. Therefore, only a residual amount of β_2M (0−300 μg L^{-1}) is excreted.[36]

https://doi.org/10.1021/acsbiomaterials.2c00036
ACS Biomater. Sci. Eng. 2022, 8, 3142−3161

Table 1. Versatile Application of β_2M as Biomarkers in Multiple Medicine Disciplines[a]

ref	study design	targeted populations/application	β_2M assay sampling	biomarker type	study outcome/remarks
			Pediatrics		
30	prospective	96 extremely preterm infants	urinary β_2M at birth	susceptibility/risk	High urinary β_2M at birth is indicative of fetal inflammatory response, and patients are prone to develop bronchopulmonary dysplasia.
45	retrospective	59 very preterm and extremely preterm infants	CSF β_2M	diagnostic	β_2M shows higher Sn and Sp than leucocytes in detecting patients with central nervous system infections and other inflammatory processes.
46	prospective	80 term infants with perinatal asphyxia	urinary β_2M	diagnostic	Urinary β_2M can be useful to predict AKI early in term neonates with perinatal asphyxia.
47	case-control	70 children with beta thalassemia major and 20 healthy controls	serum β_2M	monitoring	The Sn and Sp of β_2M are higher than creatine in capturing small eGFR changes for monitoring glomerular and tubular dysfunction in thalassaemic children.
			Geriatrics		
36	prospective	1034 nondisabled elderly aged 65 years old and above	serum β_2M	susceptibility/risk	Serum β_2M is an independent predictor of total mortality in the elderly population, and its performance precedes cystatin C and CRP.
48	cross-sectional	1663 elderly adults aged 70−84 years old	serum β_2M	susceptibility/risk	Serum β_2M was significantly associated with both frailty phenotype and index among the elderly Chinese population.
			Cardiology/Endocrinology/Nephrology/Radiology		
49	systematic review and meta-analysis	16 studies involving 30988 participants and 5391 cardiovascular diesase events	serum β_2M	prognostic	β_2M level is moderately associated with cardiovascular disease outcome and event mortality.
50	observational/prospective	872 patients admitted with acute coronary syndrome and underwent coronary angiogram	serum β_2M	susceptibility/risk	β_2M is an independent risk factor of coronary stenosis in patients with acute coronary syndrome whereby an elevated level positively correlated with Gensini score.
51	observational/prospective	1762 patients with either background of CAD or underwent coronary angiogram	serum β_2M	diagnostic and prognostic	β_2M is suggested as a biomarker for CAD for its significant association. β_2M is also positively associated with the imaging scoring, Gensini score, and SYNTAX score system.
52	prospective	366 patients with T2DM and preserved renal function with no clinical evidence of cardiovascular disease	serum β_2M	prognostic	Higher serum β_2M was an independent risk factor for subclinical atherosclerosis and diabetic nephropathy in patients with T2DM without renal impairment.
53	observational/cross-sectional	102 diabetic patients and 103 healthy controls	urinary β_2M	prognostic	Urinary β_2M correlates well with urinary albumin concentration and could be useful in detecting early nephropathy in T2DM.
54	observational/cross-sectional	424 patients subjected to CCTA for various reasons	serum β_2M	pharmacodynamic/response	β_2M stands superior to cystatin C and creatinine in detecting contrast-induced nephropathy from CCTA.
55	retrospective	510 patients with light chain amyloidosis treated with (ASCT)	serum β_2M	prognostic	β_2M before ASCT can be an independent predictor for overall survival in patients with light chain amyloidosis.
			Respiratory Medicine/Hematology/Neuruology		
56	case-control	30 patients with chronic obstructive pulmonary disease and lung emphysema and 21 age-matched; 76 subjects with normal lung function and non-emphysema	serum β_2M	susceptibility/risk	Serum β_2M is significantly elevated in patients with lung emphysema.
57	case-control	257 patients (123 polycythemia vera; 134 secondary polycythemia)	serum β_2M	prognostic	High β_2M, low erythropoietin, and no tobacco exposure were predictive of polycythemia vera. On the other hand, normal β_2M, normal erythropoietin, and tobacco exposure were predictive of secondary polycythemia.
58	observational/cross-sectional	CSF samples from 50 patients with Guillain−Barré syndrome (GBS) and 23 from patients with non-inflammatory neurological disorders (NINDs) as controls; plasma samples from 42 enrolled patients and 29 healthy individuals	CSF β_2M and serum β_2M	diagnostic and prognostic	CSF β_2M helps in identifying GBS patients with AIDP and indicates its clinical severity. The CSF β_2M level also correlated with CSF lactate dehydrogenase and lactate levels, whereas plasma CSF showed no statistical difference in GBS patients with AIDP.
			Infectious Disease		
59	cross-sectional	(HIV-1) infected patients treated with tenofovir	urinary β_2M	safety	Urinary β_2M can be a screening marker in the early detection of tenofovir nephrotoxicity.
61	cross-sectional	50 patients infected with HIV	serum β_2M	predictive	β_2M can predict HIV progression and correlates well with patients on highly active antiretroviral therapy.

https://doi.org/10.1021/acsbiomaterials.2c00036
ACS Biomater. Sci. Eng. 2022, 8, 3142−3161

Table 1. continued

ref	study design	targeted populations/application	β_2M assay sampling	biomarker type	study outcome/remarks
			Infectious Disease		
62	case-control	46 HBV-infected patients with positive surface antigen detected	salivary β_2M	Prognostic	Salivary β_2M level reflects HBV viral proliferation, but more studies should be conducted to validate salivary β_2M level further.
63	prospective	58 adults infected with human African trypanosomiasis	CSF β_2M	prognostic	The CSF β_2M level is more superior to osteopontin in discriminating the stages of human African trypanosomiasis. Still, the combination of both biomarkers yields higher Sn and Sp.
			Rheumatology/Autoimmune Disease		
64	case-control	50 patients with SLE disease and 25 healthy individuals	serum β_2M	monitoring	The serum β_2M is significantly high in SLE patients and correlates with 24 h urine protein, ESR, disease activity score, and CRP.
65	case-control	100 SLE patients and 50 healthy controls	serum β_2M	monitoring	Serum β_2M correlates well with anti-dsDNA antibody, hemoglobin, complement, and SLE disease activity index changes.
66	prospective	395 patients primary Sjögren's syndrome follow-up	serum β_2M	monitoring	Serum β_2M along with free immunoglobulins' light chains are both associated with increased systemic disease activity.
67	case-control	87 UC patients, 74 with CD, and 68 healthy control subjects	serum β_2M	monitoring	Serum β_2M was found elevated, particularly in CD, and reflective on the disease activity, severity, and extension. The level was not significant in disease remission.
68	prospective	43 UC patients, 35 with CD, and 30 control subjects	serum β_2M	monitoring	Serum β_2M levels were raised and similar in both UC and CD at the active stage.
			Surgery/Oncology		
69	prospective	148 patients with asymptomatic MM	serum β_2M	predictive and prognostic	Serum β_2M is an independent predictor of asymptomatic MM progression and adds value to disease prognostication.
37	prospective	12300 healthy volunteers	serum β_2M	predictive	Healthy individuals with higher β_2M have an increased risk of colorectal cancer.
60	retrospective/observational	227 patients with hematologic malignancies subjected to allogeneic hematopoietic cell transplantation	pretransplant serum β_2M	prognostic	A significant association exists between higher serum β_2M levels and disease relapse/progression resulting in poorer overall survival.
			Public Health		
70	prospective	3139 inhabitants (1404 men and 1735 women) in a Cd-polluted area	urinary β_2M	predictive and prognostic	In Cd-polluted area, high urinary β_2M levels result in higher mortality rate and are associated with higher risks of circulatory disease, digestive system diseases, and nephrotic diseases in men and women, and with senility for women.

[a]AIDP, acute inflammatory demyelinating polyneuropathy; ASCT, autologous stem cell transplantation; CAD, coronary artery disease; CCTA, coronary computed tomography angiography; Cd, cadmium; CD, Crohn's disease; CRP, C-reactive protein; CSF, cerebrospinal fluid; eGFR, estimated glomerular filtration rate; ESR, erythrocyte sedimentation rate; GBS, Guillain−Barré syndrome; HB,: hepatitis B virus; HIV, human immunodeficiency virus; MM, multiple myeloma; SLE, systemic lupus erythematosus; Sn, sensitivity; Sp, specificity; T2DM, type 2 diabetics mellitus; UC, ulcerative colitis.

https://doi.org/10.1021/acsbiomaterials.2c00036
ACS Biomater. Sci. Eng. 2022, 8, 3142−3161

Table 2. Correlation of Age on β_2M Studies

study no.	targeted population	age group	equation[a]	correlation coefficient	correlation strength	ref
1	1131 healthy children (504 boys and 627 girls)	0−17 years old	$\beta_2M/(mg\ L^{-1}) = -0.000472(age)^3 + 0.0139(age)^2 - 0.149(age) + 1.94$	−0.50	moderate	78
2	174 children (113 male and 61 female) with chronic CKD	1 month old−18 years old	$\beta_2M/(mg\ L^{-1}) = -0.0341(age) + 1.72$	−0.47	moderate	74
3	107 samples (96 children and 11 young adults)	2−21 years	$\beta_2M/(mg\ L^{-1}) = 1.57 - 0.01(age)$	0.02	negligible	72
4	119 healthy individuals (33 male and 86 female)			0.188	weak	75

[a]Age measured in year unit.

Hemodynamics governs the production and elimination of β_2M to preserve a reasonably constant concentration of serum β_2M in healthy individuals. Therefore, elevated β_2M levels indicate increased cell turnover or reduced kidney function. Elevated β_2M levels have been noted in multiple pathological states, such as autoimmune diseases, infections, malignancies, and renal dysfunction.[31,36,37] This will be further discussed in the following section.

1.4. Hemodynamic Imbalance. Because β_2M plays a critical role in immune surveillance and modulation, its synthesis is heightened in response to systemic inflammation due to infection and in autoimmune diseases, such as systemic lupus erythematosus, inflammatory bowel diseases such as Crohn's disease and ulcerative colitis, and Sjörgen's syndrome, to name a few. Therefore, β_2M concentrations reflect active lymphocyte proliferation.[38] Because it is a component of the MHC, an inflammatory response upregulates β_2M expression in parenchymal and interstitial cells at all sites.[30] This results in an overproduction of serum β_2M, where the immune activation exceeds the tubular reabsorption threshold. Because an inflammatory response may simultaneously injure the proximal tubule, renal reabsorption is further impaired, resulting in increased urinary β_2M excretion.

This is also evident among infants with high urinary β_2M at birth, which is mainly caused by an intrauterine inflammatory response irrespective of gestational age. As such, urinary β_2M at birth indicates both the intensity of the inflammatory response and the degree of tubular damage.[30] A systemic activation of the fetal immune system has been detected in multiple cases of fetal inflammatory response syndrome. According to the Barker fetal programming hypothesis, the excessive production of pro-inflammatory cytokines leads to organ damage resulting in long-term adverse outcomes.[39] Because β_2M cannot cross the placental barrier, studies suggest that determining the amount of β_2M present in cord blood could help assess renal function in feti.[31]

In oncology, β_2M is extensively involved in the functional regulation, i.e., survival, proliferation, invasion, migration, angiogenesis, apoptosis, and metastasis, of cancer cells.[40,41] High levels of β_2M in bodily fluids are a significant indicator of tumor burden and prognosis. Increased β_2M concentrations have been noted in esophageal cancer, multiple myeloma, renal cell carcinoma, ovarian cancer, and prostate cancer.[3,32,42] In order to avoid immune surveillance, these neoplastic cells often downregulate the expression of MHC-I via free β_2M secretion. When less β_2M is expressed on the surface of tumor cells, they successfully evade phagocytosis via macrophages and immune responses.[32,42] Moreover, β_2M facilitates the invasion and metastasis of cancer cells by acting as a growth-promoting factor and signaling molecule.[42,43] Meanwhile, the function of β_2M as a negative growth regulator depends on the type of cell and mainly involves hematological malignancies, particularly in myeloid and leukemic cell lines.[40,41,43] However, because the underlying mechanisms are often complicated and highly variable, further studies are required to explore these sophisticated mechanisms.

Various pharmacological agents have been found to affect β_2M levels. Becasue the kidneys are highly susceptible to drug-related injuries from contrast agents and nephrotoxic drugs, such as cisplatin, cyclosporine, and gentamicin,[38] acute kidney injury impairs drug uptake and increases urinary β_2M. However, patients receiving dose-dependent glucocorticoid treatment have exhibited reduced β_2M levels.[31]

2. ROLE OF β_2M AS A BIOMARKER

A biomarker can be described as "a defining characteristic that is measured as an indicator of normal biological processes, pathogenic processes, or responses to exposure or intervention".[44] Table 1 provides the medical significance of β_2M as a remarkable biomarker in numerous medical disciplines, including pediatrics, geriatrics medicine, oncology, internal medicine, infectious disease, and even surgery.

However, the impact of sociodemographic factors on β_2M is still a point of contention. Of these factors, age is the most commonly evaluated factor, while little light has been shed on the influence of gender and race. Nevertheless, a few studies have found that gender does not influence serum β_2M levels.[71,72] Table 2 shows the correlation of age in several studies across the targeted population. The interpretation of correlation strength is directly associated with the absolute magnitude of correlation coefficient: negligible (0.00−0.10); weak (0.10−0.39); moderate (0.40−0.69); strong (0.70−0.89); and very strong (0.90−1.00).[73] In the study by Ikezumi et al., a correlation coefficient of −0.50 was reported for the relationship between β_2M levels and age, indicating a moderate correlation between the two factors.[78] Further studies by the same authors reported the same correlation strength at a correlation coefficient of −0.47 despite differences in population size and health status.[74] On the other hand, a correction coefficient of 0.188 was also reported in a much broader age of healthy individuals, suggesting that β_2M levels increase with aging.[75] It can be concluded that there is no definitive reason for discrepancies in the correlation coefficient regardless of the age and health status of the subjects. In short, due to a lack of correlation between β_2M and demographic variables, β_2M can be used as a solitary marker to assess kidney function in noncommunicable diseases.

Of the multiple methods of assessing renal function, estimated glomerular filtration rate (eGFR) using creatine and cystatin C-based equations is most commonly used.

https://doi.org/10.1021/acsbiomaterials.2c00036
ACS Biomater. Sci. Eng. 2022, 8, 3142−3161

Table 3. CKD-EPI β_2M-Based eGFR Equations

study no.	CKD-EPI of β_2M-based eGFR equation [eGFR], (mL/min)/(1.73 m^2); [β_2M], mg L^{-1}	R^{2a}	age group	nationality	ref
1	$eGFR_{\beta_2M} = 10^{2.00-\log \beta_2M/0.89}$	−0.94	adult	Swedish	80
2	$eGFR_{\beta_2M} = 10^{0.96-\log \beta_2M/3.12}$, [$\beta_2$M] < 2.45	−0.996	adult	French	81
	$eGFR_{\beta_2M} = 10^{0.53-\log \beta_2M/0.76}$, [$\beta_2$M] > 2.45	−0.98			
3	$eGFR_{\beta_2M} = e^{5.27-\ln \beta_2M/0.95}$	−0.79	adult	Swedish	82
4	$eGFR_{\beta_2M} = 10^{1.95-\log \beta_2M/0.86}$	−0.971	adult	American	79
5	$eGFR_{\beta_2M} = \frac{\frac{1}{\beta_2M} - 0.144}{0.006}$	0.731	adult	Italian	83
6	$eGFR_{\beta_2M} = e^{4.279-\ln \beta_2M/0.814}$	0.90	adult	Italian	84
7	$eGFR_{\beta_2M} = e^{\ln(\beta_2M/49.406)/-0.8097}$	0.9238	adult	Italian	85
8					
b	$eGFR_{\beta_2M}{}^{b} = 104.8 \times \beta_2M^{-0.9686}$	0.9394	adult	Italian	86
c	$eGFR_{\beta_2M}{}^{c} = 107.1 \times \beta_2M^{-0.9815}$	0.9345			
9	$eGFR_{\beta_2M} = \frac{149.0}{\beta_2M} + 9.153$	0.86	pediatric, adult	Japanese	74
10	$eGFR_{\beta_2M} = \frac{160.3}{\beta_2M} - 4.2$	0.67	adult	British	87
11	$eGFR_{\beta_2M} = 133 \times \beta_2M^{-0.854}$	-		American	77

[a] R_2, regression coefficient . [b] For women. [c] For men.

However, because these traditional markers have limitations, it spurs the search for alternative markers.[3,76] Numerous studies have suggested the use of β_2M as an indicator of renal function and the potential use of β_2M-based eGFR equations. However, β_2M may be more suited to evaluating eGFR in adults instead of the pediatric population as it provides mixed results in the latter group.[3]

The eGFR can be characterized using the chronic kidney disease epidemiology collaboration (CKD-EPI) equation. This statistical aggression model represents the sample group of a particular gender, ethnicity, or health condition. However, most studies report no additional advantages over the traditional equations.[3,76] Table 3 presents the CKD-EPI of β_2M-based eGFR equations from various studies. Because a fit equation was successfully obtained, studies 2, 5, and 7 were excluded. It is noteworthy that Vincent et al. came within a range constraint,[82] while only the latest of the three separate studies published by Donadio et al. was taken into consideration.[83,85,86] Two equations were considered in study 8. Hence, a total of nine equations were considered.

First, 30 points were reconstructed on the basis of the above equations. This was accomplished by using a range of β_2M concentrations of 1−30 mg L^{-1} in the equations involved. The eGFR by β_2M graph of multiple equations was then plotted (Figure 2a). Figure 2b shows a box chart plot for a range of eGFR versus the several equations as listed in Table 3. One-way analysis of variance (ANOVA) of the short-listed equations showed no significant differences in the population mean (p = 0.07). The findings suggest that nationality is an independent factor in determining eGFR. The best fit line was obtained with an R^2 of 0.900. Therefore, a global fit CKD-EPI β_2M eGFR equation was achieved (eq 1). This equation can be utilized as a general assessment across different nationalities and countries and helps reduce the gap between case studies across the globe.

$$eGFR = 355e^{-\beta_2M/0.817} + 60.7e^{-\beta_2M/5.42} \quad (1)$$

3. β_2M ASSAY DETECTION TECHNOLOGIES

The introduction of the concept of biomarkers in the medical field in the 1980s has significantly facilitated clinical decisions and management. Its application spans various fields: from molecular, physiologic, and histologic to radiographic characteristics in therapeutic interventions. In order to meet specific purposes, biomarkers can be classified into subcategories that define their putative purposes, such as diagnostic, monitoring, pharmacodynamic, predictive, prognostic, safety, and susceptibility/risk. However, it is difficult to identify biomarkers as they are often present in very low concentrations and alongside various other proteins. As such, the detection of biomarkers at very low concentrations is difficult and time-consuming in many cases. It is noteworthy that diseases that have been detected in the early stages of development are typically treated with a great probability of success.

Although multiple biomarkers can be used to indicate a particular disease, their proximity classification, be it primary, secondary, or tertiary, can be used to differentiate their specificity to the disease in question.[88] The efficacy of detection using a biomarker depends on the interaction between the characteristics of the biomarker and the measurement method. One of the drawbacks of biomarker detection is that biomarkers are often present in low

https://doi.org/10.1021/acsbiomaterials.2c00036
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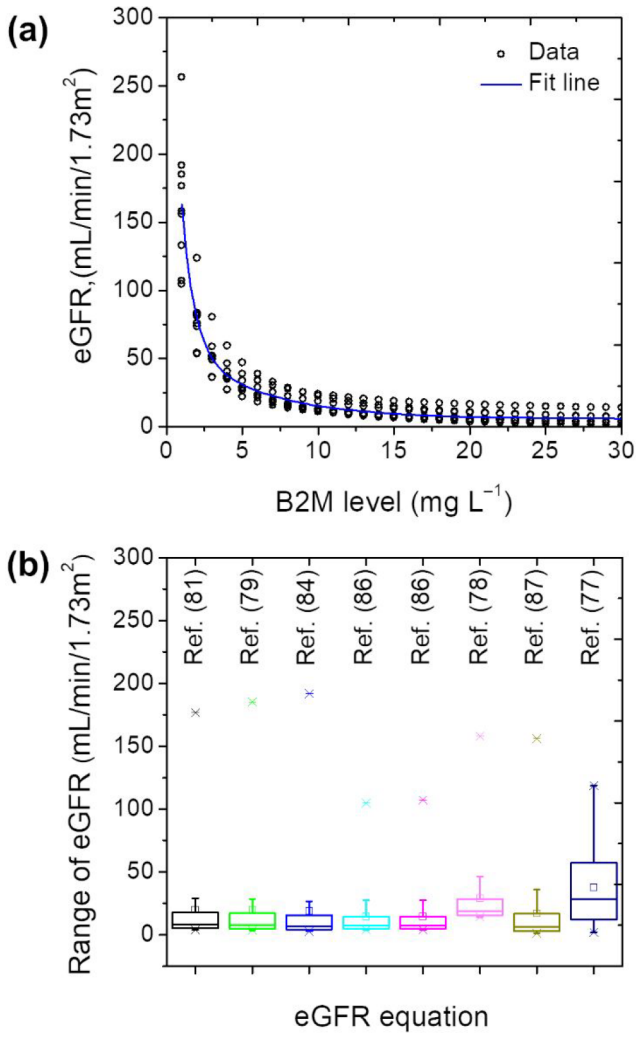

Figure 2. Chronic kidney disease epidemiology collaboration (CKD-EPI) of β_2M-based eGFR adopted from previous studies. (a) Global fit eGFR as a function of β_2M level from multiple equations. (b) Range of eGFR using different equations from the existing works.

concentrations and mixed with other proteins that increase the noise level, thereby making their detection more challenging and time-consuming.[89] To that end, the limit of detection (LoD), an analytical parameter, can be used to determine the minimum concentration required to produce a positive signal. Other factors such as specificity, sensitivity, precision, sample type and volume, and measurement method should be taken into consideration.[88] Although biomarker concentration is usually proportional to disease progression, these biomarkers need to have a long half-life in order to examine past diseases.

As mentioned earlier, β_2M has the potential for becoming versatile biomarkers for various diseases. However, misinterpretation is possible to occur if a patient has multiple diseases related to the level and fluctuation of β_2M. Furthermore, certain cancer diseases, including multiple myeloma, lymphoma, and leukemia, demand the sensitive detection of β_2M at ultralow concentrations as a critical prerequisite of an early stage of diagnostics. To address this, the disease location and the amount of β_2M in the blood, urine, or cerebrospinal fluid should be taken into consideration. An ideal versatile biomarker should have the ability to diagnose multiple diseases compared to normal healthy controls. One method to achieve this is by the combination of β_2M and other related biomarkers (e.g., β-trace protein, kidney injury molecule 1, lysozyme C, hemoglobin subunit β, immunoglobulin heavy constant α 1, serpin family F member 1, or tumor markers), as used for predicting renal disease progression,[77,90,91] renal cell carcinoma,[92] and tumor progression.[93]

3.1. Chromatography, Colorimetry, and Immunoassay Methods. Assays can be generally classified into four basic principles: physical assays, chemical assays, immunoassays, and bioassays.[94,95] However, these can be further expanded into distinctive combinations, such as physicochemical, immunochemical, and immunophysical to name a few.[94,96] Although analytical methods, such as colorimetry and chromatography, are widely used to test analytes, quantitative immunological methods, such as immunoassays, are gaining considerable attention due to their unprecedented advantages such as high specificity with detection limits measured in pico- or attomoles and applicability to a wide variety of compounds of interest.[97] More precisely, the immunoassays aim to quantify a specific single molecule in a solution on the basis of the specific immuno-reaction between an antibody and an antigen. All immunoassays are directly dependent on the complex formation that enables an antibody and an antigen to fit each other perfectly. Therefore, high-specificity antibodies and detection methods can be demonstrated even in complex biological samples such as urine, blood, or plasma.

On the other hand, colorimetry focuses on the interaction of light by quantifying the perception of color. Because it is low cost, fast, convenient, and portable or requires only simple instruments, it has been widely used for biochemical analysis and the development of biosensors. However, colorimetry is generally less sensitive as compared to immunoassays. Chromatography is a multifaceted separation method that is extensively used to separate the components of a mixture in chemical laboratories. The most common types of chromatography use paper, thin layer, and column chromatography followed by high-performance liquid chromatography (HPLC), adsorption chromatography, affinity chromatography, size exclusion chromatography (SEC), ion-exchange chromatography (IEC), and gas chromatography.[98] Although chromatography is highly sensitive and versatile and caters to a wide range of sample conditions, some chromatography methods are expensive and require preliminary analysis of the extract and other nonspecific factors to reduce susceptibility to interferences.

Immunoassays play a critical role in various analytical bioassays settings, such as biomedical, clinical chemistry, clinical diagnostics, environmental monitoring, security, and food testing due to their inherent specificity, high-throughput, and high sensitivity for the analysis of a wide range of analytes in biological samples. The recent progress of immunoassays has been significantly facilitated by the most effective and suitable analytical methods, the rapid development and refinement of many new immunoassay reagents and systems, and access to convenient and reliable commercial kits.[97] Various types of immunoassays have been developed from the numerous labeling substances that have been used to advance immunoassays. These substances include enzymes (enzyme- or enzyme), radioisotopes (radio-), bioluminescent compounds (lumino-), and fluorescent (fluoro-). The terms in parentheses indicate the common combinations. However, many aspects warrant further consideration in order to set up an immunoassay (Figure 3).[100]

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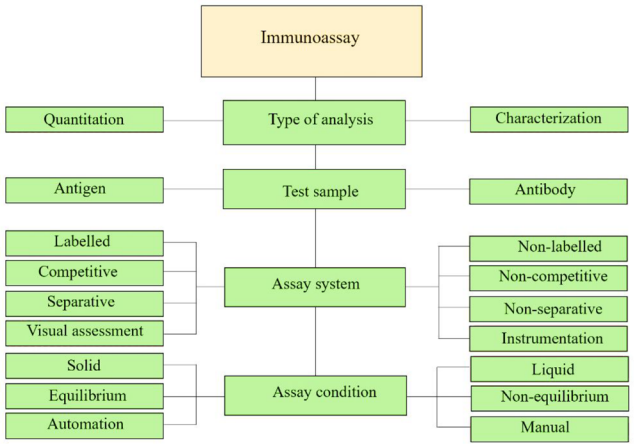

Figure 3. Classification of immunoassay.

Although colorimetry is economical, rapid, and convenient and visible changes can be qualitatively or semiqualitatively identified by the naked eye,[99,101] it is generally less sensitive than immunoassays, which are a proven highly sensitive biomarker detector.[102,103] Therefore, combining both methods to conduct novel colorimetric−immunoassays is a promising route of overcoming the limitations and increasing the biomarker detection capabilities of each individual method without compromising their simplicity and high efficiency. The stability improvement and signal amplification in colorimetric−immunoassays can be achieved by using various novel nanomaterials that enhance the coupling efficiency between the antibody and the target analyte.[104]

3.2. β_2M Detection. Protein biomarkers, such as deoxyribonucleic acid (DNA), mRNA (mRNA), antigens, and enzymes, as well as lipids, sugars, and cell-based biomarkers are the most common biomolecules used in the medical field.[89] Apart from the previously discussed methods of biomarker detection, other methods include surface plasmon resonance (SPR),[105,106] enzyme-linked immunosorbent assay (ELISA),[107,108] spinning-disk microinterferometry,[109] surface-enhanced Raman spectroscopy (SERS),[110,111] electrochemical assay,[112,113] and gel electrophoresis.[114] However, the potential of biomarkers has not been thoroughly explored due to the various limitations of the technologies currently available for biomarker detection.

Table 4 summarizes the comparison of various methods available for β_2M detection. The concentrations of β_2M in serum or urine are generally measured by conventional assays such as nephelometric immunoassay, radioimmunoassay (RIA), ELISA, latex immunoassay, and turbidimetric immunoassay. Nevertheless, the analytical performance of these methods varies in terms of sensitivity, specificity, and precision. In past decades, it has been demonstrated that RIA method can accurately examine the levels of β_2M in normal serum or urine without sample preconcentration.[120] However, it possesses several drawbacks such as short shelf life, high cost, health hazards, and time-consuming process. As an alternative to the RIA method, commercially accessible ELISA or latex kits have proven to be beneficial in identifying possible diseases associated with β_2M and other related proteins due to their simplicity to create and use in many applications. A simple and robust method by ELISA and latex-based assay show a higher sensitivity for β_2M, with LoD values in the sub-

Table 4. Comparison of Various Methods Available for β_2M Detection[a]

method of detection	detection range	limit of detection	fluid sampling	application	ref
gel filtration chromatography	0.10−0.30 mg mL^{-1}	0.08 mg mL^{-1}	β_2M in PBS	β_2M industrial production	116
high-performance liquid chromatography	0.2−5.0 μg mL^{-1}	0.058 μg mL^{-1}	serum	clinical diagnosis	117
MALDI-MS	10−50 μg mL^{-1}		serum	detection of β_2M and its glycosylated form	119
MSIA	0.010−1.0 μg mL^{-1}	2.5 μg mL^{-1}	tears, saliva, plasma, and urine	classification of β_2M and its glycosylated form	118
immunoassays based on HECL	1−1000 μg mL^{-1}		bovine serum	clinical diagnosis	115
RIA	1.2−2.2 μg mL^{-1}	0.64 μg mL^{-1}	serum	renal disorders	79
RIA	∼1−100 ng mL^{-1}		urine, serum, saliva, and colostrum	clinical diagnosis	120
RIA	0.039−0.109 μg mL^{-1}		bovine urine	clinical diagnosis	121
ratiometric immunoassay	0.25−8.0 μg mL^{-1}	0.17 μg mL^{-1}	serum	clinical diagnosis	122
latex immunoassay	1−32 ng mL^{-1}	0.5 ng mL^{-1}	urine and serum	clinical diagnosis	123
nephelometric immunoassay	3.8−38 μg mL^{-1}		urine	monitoring kidney transplant patients	130
particle-enhanced nephelometric immunoassay	0.3−40 μg mL^{-1}	0.3 μg mL^{-1}	serum	clinical diagnosis	131
turbidimetric immunoassay	0.4−16 μg mL^{-1}	0.4 μg mL^{-1}	serum	myelomatosis	128
turbidimetric immunoassay	73−2156 ng mL^{-1}	∼13.2 ng mL^{-1}	urine	clinical diagnosis	129
PETIA	0.2−40 μg mL^{-1}	0.2 μg mL^{-1}	serum	clinical diagnosis	127
ELISA	0.2−22 ng mL^{-1}	0.02 ng mL^{-1}	urine	urinary and fractional excretion	124
ELISA	0.44−14 ng mL^{-1}	0.440 ng mL^{-1}	serum	myelomatosis	125
ELISA	0.62−3.28 μg mL^{-1}	0.212 ng mL^{-1}	cerebrospinal fluid	central nervous system disorders	126
dual-label TRFIA	20−1000 ng mL^{-1}	1 ng mL^{-1}	serum	lymphocytic leukemia	132
dual-label TRFIA	2−1000 ng mL^{-1}	2.13 ng mL^{-1}	urine	contrast-induced nephropathy	133
microcapsule-based immunoassay	10^{-3}−10^{5} pg mL^{-1}	7.7 fg mL^{-1}	human β_2M	clinical diagnosis	134

[a]ELISA, enzyme-linked immunoassay; HELC, hot electron-induced electrochemiluminescence; MALDI-MS, matrix-assisted laser desorption−mass spectrometry; MSIA, mass spectrometric immunoassay; PBS, phosphate-buffered solution; PETIA, particle-enhanced turbidimetric immunoassay; RA, radioimmunoassay; TRFIA, time-resolved fluorescence immunoassay.

https://doi.org/10.1021/acsbiomaterials.2c00036
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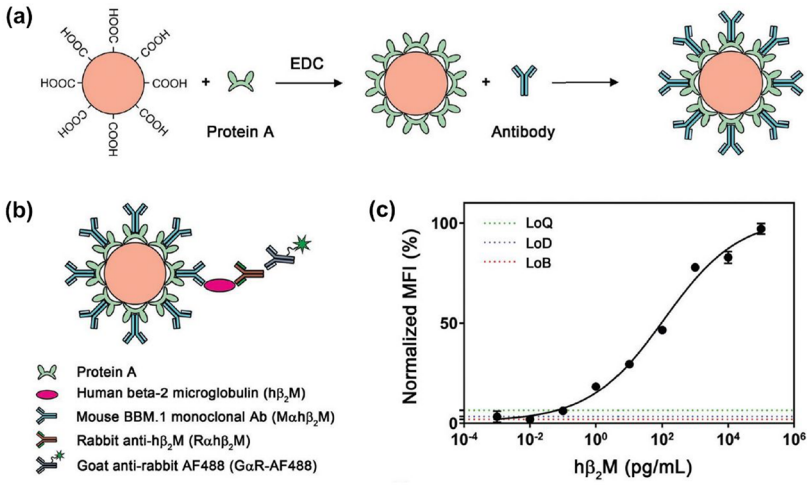

Figure 4. Microcapsule-based sandwich assay for detection of proteins and nucleic acids. (a) Biofunctionalization schematic indicates protein A-based approach of antibody immobilization on the microcapsule surface. Protein A is covalently attached onto the activated surface of the carboxylic acid groups for oriented binding of antibodies. (b) Schematic representation of hβ_2M detection. (c) Detection of hβ_2M in PBS. The limits of blank (LoB), detection (LoD), and quantification (LoQ) indicated by dotted lines are interpolated from the corresponding calibration curves. Reproduced with permission from ref 134. Copyright 2016 John Wiley & Sons.

ng mL^{-1} range,[121,124−126] while the other methods, including RIA, nephelometric immunoassay, and turbidimetric immunoassay for β_2M, have LoD values in the sub-μg mL^{-1} range.[79,127−130]

Although the conventional assays can cover the clinically useful range of concentrations in the blood, only trace amounts of β_2M (less than 360 μg L^{-1}) can be normally detected in urine as most of it is absorbed by the kidneys.[3] Furthermore, urine testing for β_2M is not frequently performed due to the complexity of testing procedures. Because only a trace amount of β_2M passes through the kidneys, highly sensitive and selective techniques that can detect β_2M through a simple urine test are crucial for addressing this unmet diagnostic need. Therefore, a recent microcapsule-based sandwich assay was established to enable a simple and rapid quantitative measurement for β_2M, while providing high sensitivity and selectivity at very small sample quantities.[134,135] The assay consists of microcapsules that are chemically cross-linked and coated with protein A before binding with antibody (Figure 4a). The principle of human β-2 microglobulin (hβ_2M) detection using protein A-coated microcapsules in a sandwich-like assay format is based on the changes in fluorescence signal when the hβ_2M is relatively sandwiched between a binder and a detector molecule (Figure 4b). From the hβ_2M dose response measurements (Figure 4c), the microcapsule-based assay enables ultrasensitive hβ_2M detection at 1 pg mL^{-1} with saturation of the signal at 10 ng mL^{-1}, giving the highest sensitivity for hβ_2M with a LoD in the fg mL^{-1} range.

Digital measurement methods such as digital enzyme-linked immunosorbent assay (dELISA) (or single-molecule arrays technology) and digital polymerase chain reaction (dPCR) enable ultrasensitive detection of very low concentrations of biomolecules with sub-femtomolar or even attomolar sensitivity.[136−138] Recent emerging digital ELISA platform based on molecular-on-bead signal amplification for individual counting (MOSAIC) achieves a low-attomolar limit of detection, with an order of magnitude enhancement in sensitivity over the state-of-the-art methods.[139] Interestingly, multiple proteins can be measured simultaneously for higher-order multiplexing with femtomolar sensitivities or below, compared with microwell- or droplet-based digital methods. By measuring very low concentrations of biomolecules such as β_2M and other related proteins that are undetectable by current methods, the digital ELISA platform based on MOSAIC provides a highly accurate detection that can potentially improve diagnostics and prognostics for applications such as kidney and cancer diseases.

4. β_2M BIOSENSOR

Expensive and sophisticated tools require high maintenance, labor cost for trained laboratory personnel to perform sample preparations and run analyses, and long turnaround times while clinical diagnostic tests are highly undesirable as they are often tax-consuming and not widely accessible. However, these limitations can be overcome with emerging point-of-care or home-care tests that adopt a "sensing" method that does not compromise detection sensitivity. An ideal sensor converts the desired input to readable electrical output. The sensors can be classified as physical sensors, chemical sensors, or biosensors. In recent years, β_2M biosensors have come under scrutiny due to high demand from the medical community for unprecedented performance, mobility, immediate result, and lower cost. The main components in a biosensor are typically an analyte, a bioreceptor, and a transducer. The analyte acts as an agent to be captured and detected by the bioreceptor, while the transducer converts the signal changes from the captured activity to readable data.

4.1. DNA-Based Biosensors. DNA-based biosensors play a critical role in different fields, such as environmental monitoring, food control, drug discovery, forensics, and biomedical research. In a DNA-based biosensor, the biological reaction that occurs between the bioreceptor and the target analyte produces or consumes ions or electrons. This changes the electrical current, potential, or other electrical properties of the solution. A transducer then converts this biological signal into a detectable electrical signal proportional to the target concentration and displays it on a computer screen.[140] The most important benefits of DNA-based biosensors are that they provide reliable results and rapid testing and are low cost. However, DNA-based biosensors are not without their limitations. In general, the materials of a DNA-based biosensor

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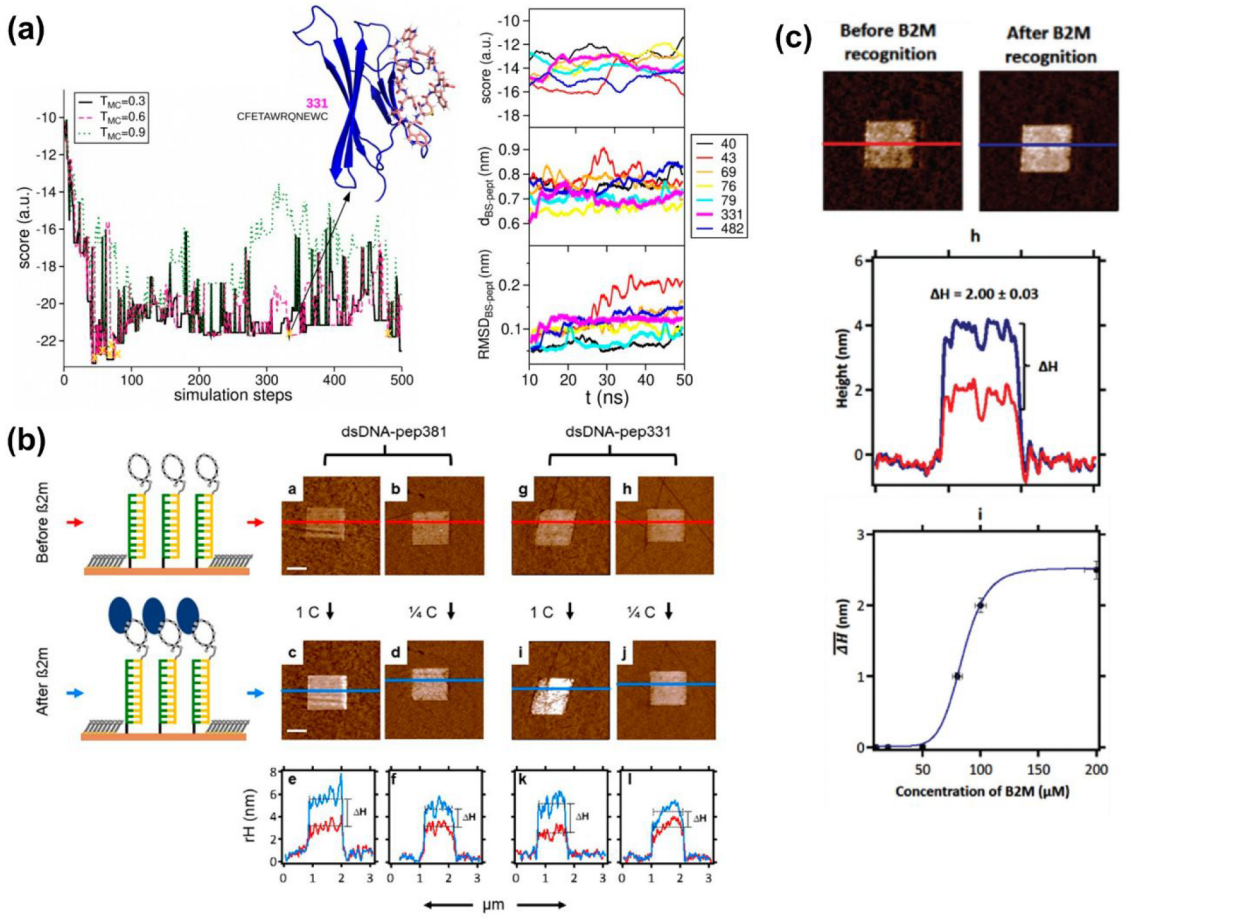

Figure 5. β_2M biosensors based on DNA conjugation method. (a) Computational peptide design showing protocol for amino acid refinement through computational evolution (PARCE) optimization and screening of selected peptides based on three descriptors: binding score; distance between peptide and protein binding site; and root-mean-square deviation (RMSD) of the backbone of both β_2M and peptide. The β_2M structure with PDB code 1LDS was used for the computational studies. (b) AFM detection and quantification of the recognition of β_2M by dsDNA−pep381 and dsDNA−pep331. The corresponding differential height profiles showing their respective binding response of both dsDNA−pep381 and dsDNA−pep331 nanopatches toward the β_2M. Reproduced with permission from ref 142. Copyright 2021 The Authors, under Creative Commons CC BY 4.0 license, published by Multidisciplinary Digital Publishing Institute. (c) AFM measurements with high-resolution imaging showing the change in height associated with β_2M−cyclic peptide interaction. Reproduced with permission from ref 144. Copyright 2017 Royal Society of Chemistry.

must interact with the DNA on an atomic level because a DNA-based biosensor can only identify materials that interact with the DNA. In fact, the nanomaterials must bind strongly to the DNA under uncontrolled conditions. However, finding these nanomaterials poses a challenge in the application of DNA-based biosensors.[141]

The DNA conjugation method of capturing target analytes is an alternative technology to the immunoassay approach. Recently, Fortuna and co-workers developed an ssDNA−peptide conjugate-based β_2M biosensor using computational and experimental methods.[142,143] The binding sites in silico were differentiated using protocol for amino acid refinement through computational evolution (PARCE) simulations, and the combination of AutoDock Vina, LIGPLOT, and Gromacs was used to screen the efficacy of 20 different peptide sequences (Figure 5a). On the basis of the optimal behaviors of three descriptors, the pep381 peptide structure with the CRRYSHQHYRHC sequence and the pep331 peptide structure with the CFETAWRQNEWC sequence were selected against binding sites A and B for the atomic force microscopy (AFM) characterization, respectively.[142]

Figure 5b illustrates how AFM was utilized to perform high-resolution imaging and characterize the recognition of the β_2M by the peptide using the quantitative method. To demonstrate the capability of AFM in capturing β_2M, the thiol-modified dsDNA (green)−peptide was conjugated (yellow) with molecules bonded on ultraflat gold (Au(111); orange) via DNA-directed immobilization (DDI) and surrounded with a bioresistant top-oligo-ethylene glycol (TOEG) monolayer. Because the differential height upon binding (ΔH) of the β_2M was almost comparable, it proved the presence of both peptides albeit at different concentrations (C). Therefore, because the dsDNA−pep381 and dsDNA−pep331 behaved similarly, they were both capable of capturing their respective binding sites on the β_2M with relatively high sensitivity. Another study by Fortuna and co-workers showed constant changes in height (ΔH = 2 nm), indicating the cyclical binding signal of the peptide to the β_2M in a standard solution (Figure 5c).[144] It is noteworthy that ΔH tends to saturate in solutions containing a β_2M concentration of more than 100 μM. Although AFM can itself act as a biosensor, it struggles to

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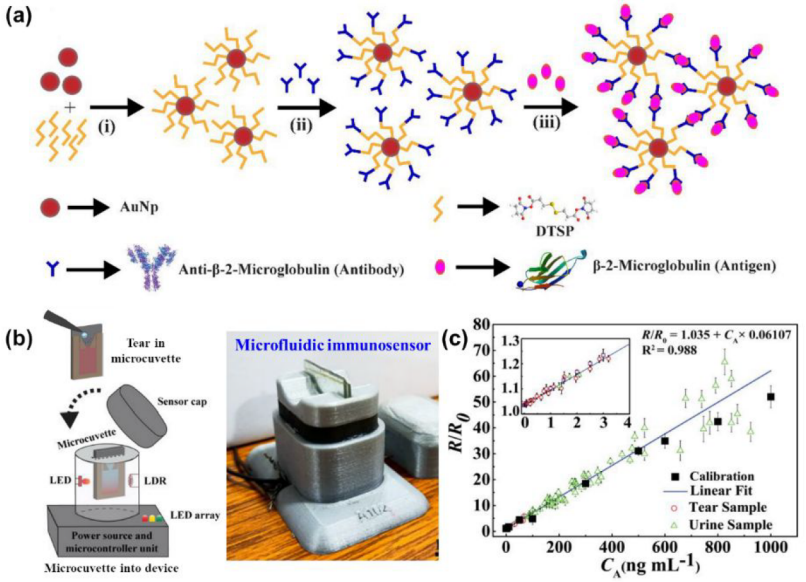

Figure 6. Detection of β_2M in human tears by microfluidic immuno-sensor. (a) Schematic representation of the preparation of water-based suspended Au nanoparticles-anti-β_2M conjugates for the β_2M binding. (b) Microfluidic immuno-sensor device with an insertion of microcuvette utilizing LED and LDR measurements for the detection of β_2M in human tears. (c) Normalized resistance response with the concentration of β_2M from zero to 1000 ng mL^{-1} in tear and urine samples. Reproduced with permission from ref 35. Copyright 2020 American Chemical Society.

differentiate between bioparticles of similar sizes in a complex system.[145]

4.2. Immuno-based Biosensors. An affinity-based biosensor integrates a biological element on a solid-state surface to facilitate a reversible biospecific interaction with the target analyte and a transducer. An affinity-based biosensor that is designed to monitor the binding process uses specific binding proteins, lectins, receptors, nucleic acids, membranes, whole cells, antibodies, or antibody-related substances for biomolecular recognition. For instance, if antibodies or antibody fragments are used as the biological element, the device is an immuno-sensor.[146] The general working principle of immuno-sensors is based on the specific immunochemical recognition of antibodies, that have been immobilized on a transducer, by antibodies in the media to produce analytical signals that dynamically vary according to the concentrations of analytes of interest. In immune-sensing, however, various transducers, such as optical, electrochemical, and piezoelectric, are used. Although immuno-based biosensors are highly sensitive and selective, have low cost, require only a small amount of a sample, and are portable for practical applications, their drawbacks include depuration from the bound label, the need for washing and separation, and increased complexity of the assay, to name a few. Furthermore, the nonspecific binding of the antibodies to molecules other than the target analytes is a major hurdle in immuno-sensor development. Other issues that have delayed the commercialization of immuno-sensors are their inability to integrate and support accessories on a single format.[147]

For β_2M detection, immuno-sensors rely on an antigen−antibody interaction with the help of a transducer to obtain the responses. As such, an immuno-based sensor needs to be ultrasensitive and highly selective and provide high-throughput detection of molecular biomarkers. In recent years, the use of anti-β_2M monoclonal antibodies (Ab_{β_2M}) has been able to successfully distinguish and bind β_2M (Ag_{β_2M}). This opens the door for novel clinical findings and treatments of β_2M-related diseases. Below is a theoretical understanding of the binding affinity constant:

At equilibrium, the equilibrium association constant is

$$K_a = \frac{[Ab_{\beta_2M}Ag_{\beta_2M}]}{[Ab_{\beta_2M}][Ag_{\beta_2M}]} \quad (2)$$

where Ab_{β_2M} and Ag_{β_2M} are the concentrations of unbound antibodies and the β_2M antigen and $[Ab_{\beta_2M}Ag_{\beta_2M}]$ is the complex compound. The total concentration of the β_2M antigen present, unbound and bound, is then

$$[Ag_{\beta_2M}(T)] = [Ab_{\beta_2M}Ag_{\beta_2M}] + Ag_{\beta_2M} \quad (3)$$

r is the number of moles of the β_2M antibody bound to the total moles of the β_2M antigen:

$$r = \frac{[Ab_{\beta_2M}Ag_{\beta_2M}]}{[Ag_{\beta_2M}(T)]} \quad (4)$$

Equations 2 and 3 are substituted into eq 4 to form

$$r = \frac{K_a[Ab_{\beta_2M}]}{K_a[Ab_{\beta_2M}] + 1} \quad (5)$$

Equation 5 holds true when there is only one binding site on the β_2M antibody, Ab_{β_2M}. If there is more than one or n number of binding sites, the equation must be rearranged into the Scatchard equation:

$$\frac{r}{[Ab_{\beta_2M}]} = nK_a - rK_a \quad (6)$$

From the Scatchard plot, K_a can be obtained from the graph gradient by plotting $\frac{r}{[Ab_{\beta_2M}]}$ versus r. Some assumptions were made to obtain eq 6.[148] The association and dissociation equilibrium constants, K_a and K_d, can be related to $K_d = \frac{1}{K_a}$;

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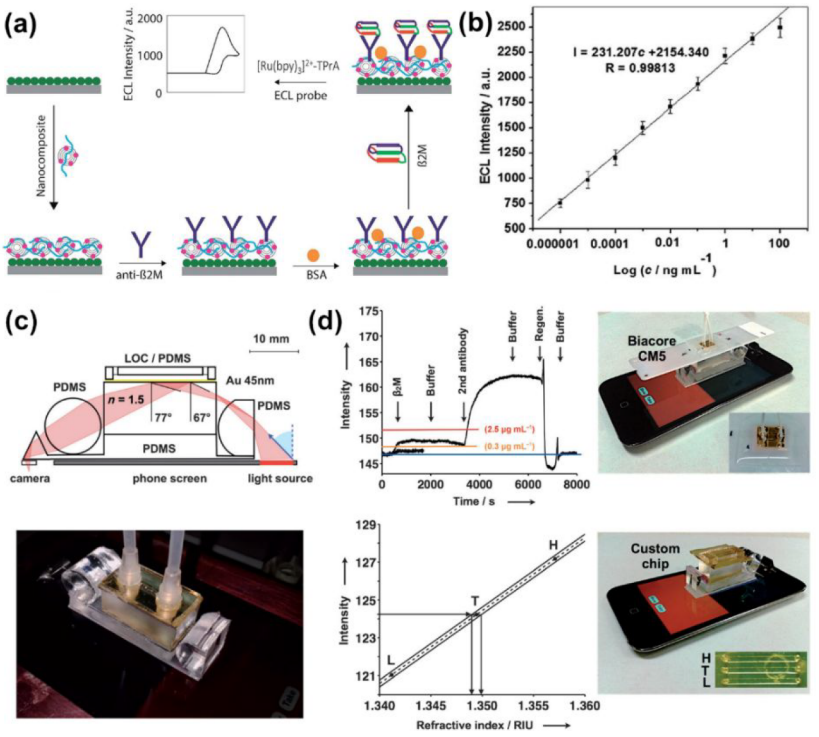

Figure 7. β_2M biosensors based on antibody−antigen interaction. (a) Schematic representation of the label-free electrochemiluminescence immuno-sensor for β_2M detection based on a AuNPs@CNOs-CS nanocomposite-modified QDs-SPE. (b) Calibration curve of the immuno-sensor at different β_2M concentrations. Reproduced with permission from ref 153. Copyright 2017 Royal Society of Chemistry. (c) Schematic representation and photograph of angle-resolved surface plasmon resonance (SPR) on a phone screen showing the light path from screen to camera for β_2M detection. (d) Interaction of β_2M and functionalized Au surface with a monoclonal mouse antibody of β_2M using a Biacore CM5 test chip and custom chip (with embedded calibrations). Reproduced with permission from ref 155. Copyright 2012 John Wiley & Sons.

the key point to convey the estimated binding affinity constant mostly refers more to K_d. The smaller the value of K_d, the stronger the bond between the antigen and antibody. K_d depends on several factors, such as temperature, pH, ionic strength, antigen concentration, antibody concentration, and incubation duration.[149] Apart from that, the method used in the binding affinity studies affects the K_d range,[150] while the use of monoclonal or polyclonal antibodies affects its interaction with the antigen. Monoclonal antibody−antigen usually has a higher binding affinity.[151] Higher binding affinity yields significant changes in electrical-, optical-, and mechanical-assisted responses and can, therefore, facilitate the sensitive detection of target analytes. In recent years, immuno-based biosensors have been extensively investigated for β_2M detection.

Maity et al. developed a microfluidic sensor mechanism that detects β_2M in human tears, which is a complex binding mixture of a water-based solution (Figure 6a).[35] The microfluidic immuno-sensor consisted of an aqueous suspension of gold nanoparticles (AuNPs) that had been immobilized with anti-β_2M antibodies using a 3,3′-dithiodipropionic acid di(*N*-hydroxysuccinimide ester) (DTSP) linker to form an AuNP-DTSP-anti-β_2M composite. This composite was then placed in a specially designed transparent glass microfluidic cuvette before the collected specimens, i.e., tears containing β_2M antigens, was added to allow the antibody−antigen reaction to occur via the binding process. Raman spectroscopy indicated that the agglomeration of the AuNP enhanced the signals from the localized surface plasmon resonance (SPR) of the AuNPs. A light scattering technique was used to transduce the colorimetric variations into electrical signals. As seen in Figure 6b, the agglutination between the antigens and the antibodies was sufficient to scatter light and cause a shift in resistance that was detectable using a light-emitting diode (LED) and a light-dependent resistor (LDR). The operating range was linear in the range of ~10^{-4} to 500 ng mL^{-1} with an R^2 value of 0.988 (Figure 6c).

Khan et al. developed an economical and different paper-based transistor to detect β_2M in tears.[152] The immobilization method used was similar to that of previous studies, where the gold (Au) electrode acted as the gate and the polymer graphene functioned as the drain terminal. Surface-enhanced Raman scattering (SERS) was first performed to produce a change in the I_G/I_D ratio with the Au electrodes. TheI_G/I_D ratio significantly decreased, from 5.42 to 2.95, in the absence and presence of β_2M, respectively. In terms of device response, the range of β_2M detection in PBS ranged between 0.001 and 10 ng mL^{-1} with an R^2 value of 0.9587.

On the other hand, Rizwan et al. developed an electrochemiluminescence (ECL) β_2M immuno-sensor.[153] The CdSe quantum dot screen-printed electrode (QDs-SPE) was first altered using AuNPs doped with a carbon nano-onions (CNOs) chitosan nanocomposite to form QDs-SPE/AuNPs@ CNOs-chitosan complex layers (Figure 7a). The CNOs were used as an anti-β_2M linker that binds with AuNPs. The anti-β_2M was then immobilized. Bovine serum albumin (BSA) was added prior to the ECL probe to prevent nonspecific binding in the real serum and urine samples. According to the ECL profiles, the detection range of the β_2M immuno-sensor was 10^{-6} to 10^{2} ng mL^{-1}, with a detection limit of 1 fg mL^{-1} (Figure 7b). In a study that was published separately, the long-term stability and device efficiency of the

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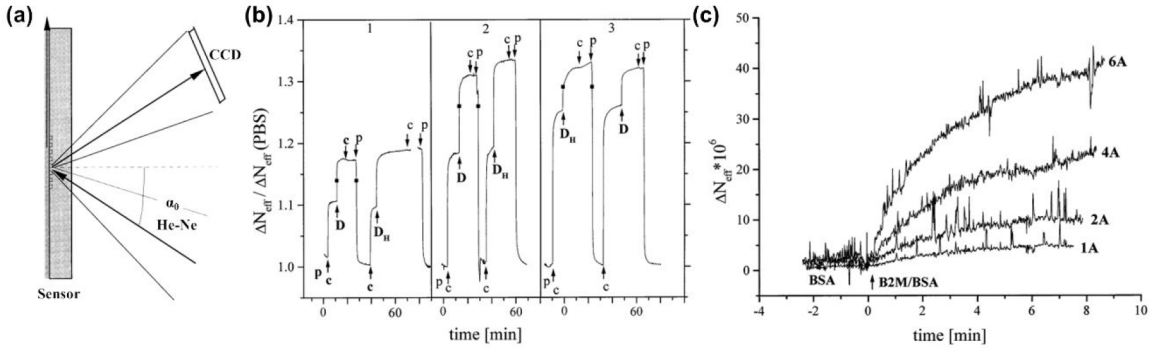

Figure 8. β_2M detection based on the reflection grating coupler (gc) sensors. (a) Measurement mechanism of the reflection gc showing the light focused from a helium−neon (He−Ne) laser on the grating to create a continuum of angles. (b) Responses of anti-β_2M three-layer networks prepared in different ways to the exchange of phosphate-buffered saline (PBS) and citrate buffer (CB) and the reabsorption of DS and DSH into the networks. (c) Response of grating coupler sensors with different numbers of immobilized anti-β_2M layers. Reproduced with permission from ref 159. Copyright 1999 Elsevier.

electrochemiluminescence β_2M immuno-sensor on days 25 and 30 were reportedly 90 and 75%, respectively. Yang et al. similarly used polyethylenimine (PEI) to enhance the ECL effect.[154]

Apart from that, Preechaburana et al. integrated an SPR setup with mobile phones to produce a disposable β_2M biosensor.[155] The angle-resolved SPR system (Figure 7c) utilizes the screen and front camera of the mobile phone for illumination and optical detection while the disposable optical coupler provided is made of poly(dimethylsiloxane) (PDMS) rubber and epoxy. A thermal evaporator was used to coat the top surface with 45 nm of Au. This surface interface carries the angle-resolved SPR signal reflected from it. The angular resolution and sensitivity of the SPR system were 0.111° pixel^{-1} and 11 × 10^{-3} refractive index unit (RIU), respectively. The performance of the SPR system was tested using two types of devices: (i) a commercial test (Biacore CM5) as a reference device for β_2M validation and (ii) a custom chip. Figure 7d depicts the interactions that were detected by the Biacore CM5 test chip and custom chip (with embedded calibrations) that had been functionalized with a monoclonal mouse antibody of β_2M. Interaction analyses were conducted for approximately 2 h and 13 min. The intensity response was noted in the absence of polyclonal rabbit anti-β_2M for the β_2M detection at 1.32 and 0.132 μg mL^{-1} concentrations, with a LoD value of about 0.1 μg mL^{-1}. The results fairly reflect the SPR platform performance and are suitable for clinical diagnosis.

Berthuy et al. developed a cell-on-chip analytical system to study prostate cancer cells by sensing β_2M and prostate-specific antigen (PSA).[156] The biosensor used an immunoassay method to immobilize anti-β_2M on an Au surface. The β_2M was quantified using surface plasmon resonance imaging (SPRi). The androgen-sensitive human prostatic cancer LNCaP cell line secretes β_2M in response to DHT induction. The SPRi measurements show broader changes in reflectivity at the anti-β_2M spot than spots with and without LNCaP on the Au surface. Lee et al. used a similar quantification method for the multiplexed detection of LMWP biomarkers,[157] while another study developed a β_2M biosensor chip mass spectrometry (BCMS) using buffer exchange surfaces aided with matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) and SPR for analysis.[158] Nedelkov et al. divided BCMS into four sections: FC1, FC2, FC3, and FC4. Each function captured the analyte by immobilizing the ligand, performing buffer exchange, protein processing, and protein processing surface. A glass slide was sputter coated with 200 nm Au. A self-assembled monolayer (SAM) of 11-mercaptoundecanoic acid as a linker was used to immobilize the anti-β_2M onto the Au surface. Prior to immobilizing the ligand, the surface was activated with 1,1″-carbonyldiimidazole to produce carboxyl groups. The flow of the buffer solution was addressed using a precise microfluidic delivery system.

Brynda et al. incorporated a unique grating coupler (GC) mechanism with a three-dimensional antibody network to detect human β_2M (Figure 8a).[159] In preparation for the anti-β_2M three-layer networks on the surface of the GC chip, the surface of the GC was subjected to repeated adsorption of anti-β_2M and dextran sulfate sodium salt (DS) and DSH that had been prepared using dextran with an average molecular weight of 5000 and 500000, respectively. This was followed by the glutaraldehyde (GA) cross-linking of the anti-β_2M and washing out the DS. Changes in the effective refractive index (ΔN_{eff}) were calculated to study the adsorption behavior of the anti-β_2M network in aqueous solutions (Figure 8b). A lower cross-linking concentration and higher molecular mass DSH interlayer junctions increased the network expansion, resulting in significant observable changes in ΔN_{eff}. Figure 8c depicts the interactions between the β_2M and the immobilized anti-β_2M at different amounts of anti-β_2M layers. The GC measurements indicated that β_2M tends to bind and diffuse into the cross-linked multilayer anti-β_2M networks, which increases the sensitivity of the GC sensor.

4.3. Nanomechanical-Based Biosensors. Nanomechanical-based biosensors are cantilever-shaped, pillar-shaped, membrane-shaped, or double-clamped beam-shaped analytical devices that quantitatively detect biomolecules. Nanomechanical biosensors generally work by translating molecular recognition of biomolecules into mechanical responses upon molecular adsorption on a surface. Changes in mechanical responses, such as force, displacement, and mass, are quantitatively measured using optical and electrical detection techniques.[160] Specifically, nano-electromechanical systems (NEMS) that have been sufficiently size-matched with the molecules that they will be interacting with can be used to develop bioaffinity-based biosensors with a single-molecule sensitivity at zeptogram scale mass resolution, hence making them unique.[161] Furthermore, as these devices experience a measurable displacement against applied force, which can be

https://doi.org/10.1021/acsbiomaterials.2c00036
ACS Biomater. Sci. Eng. 2022, 8, 3142−3161

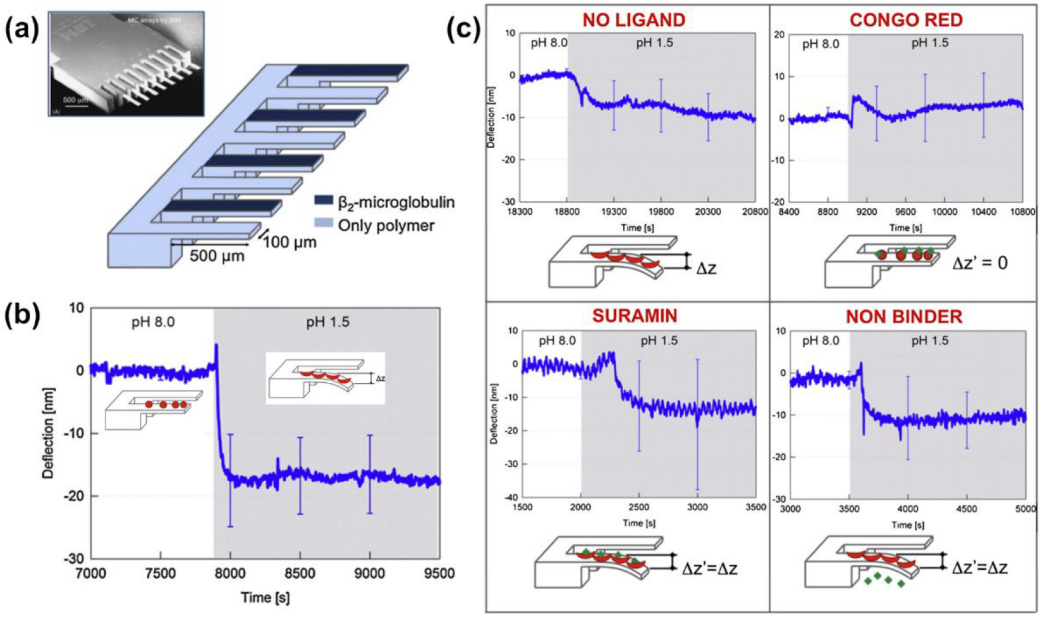

Figure 9. β_2M nanomechanical biosensors based on microcantilevers (MCs). (a) Schematic representation of the employed silicon MC arrays with β_2M and polymer only. The inset shows a scanning electron microscopy (SEM) image of the arrays. (b) MCs with β_2M responding with a specific deflection (Δz) to the pH shift, univocally probing the related β_2M conformational transformation. (c) Deflection results and schematic representations of the related binding-conformation events for the β_2M MCs incubated with no ligand (native β_2M MCs), congo red, suramin, and nonbinder. Reproduced with permission from ref 164. Copyright 2013 Elsevier.

converted into an electric signal from piezoelectric or piezoresistive effect, it can be used to quantify forces at cellular and subcellular scales.[162] However, the devices are not without outstanding challenges, such as difficulty differentially functionalizing closely packed readouts and the complexity of multiplexing the electrical readout of a dense array of devices.[163]

Olivera et al. developed distinctive nanomechanical microcantilevers (MCs) to investigate related β_2M conformational transformations.[164] The study used a total of 11 arrays of eight rectangular silicon (100) MCs, each measuring 500 μm in length, 100 μm in width, and 1 μm in thickness. Four of the eight rectangular MCs were used for β_2M functionalization, while the rest were used as controls (Figure 9a). Prior to β_2M immobilization, the silicon (Si) surfaces of the MCs were coated with copolymer via the radical polymerization of dimethylacrylamide (DMA), *N*-acryloyloxysuccinimide (NAS), and 3-(trimethoxysilyl)propyl methacrylate [co-poly(DMA-NAS-MAPS)]. Because the mechanical model cooperated, the conformational screening was conducted. The differential deflections (Δz) were measured at pH 8.0 and 1.5 because the conditions below pH 3.6 trigger the β_2M fibrillation process (Figure 9b). Following the activation of the MCs, β_2M in a phosphate-buffered saline (PBS) solution was printed on the MCs. The arrays were found to provide a response with 83% reliability and a mean differential deflection of $\Delta z = -8 \pm 2$ nm and a corresponding mean surface stress of $\sigma = -2 \pm 0.1$ mN m^{-1}. Because this study focused on investigating the chemical compounds responsible for β_2M fibrillogenesis, such as no ligand, congo red, suramin, and nonbinder, the nanomechanical results demonstrated that no differential deflection ($\Delta z = 0$) occurs for the β_2M MCs incubated with congo red as it completely suppresses β_2M conformational changes after the pH switch (Figure 9c). Among the three samples, the results suggest that only congo red is able to stabilize the β_2M in its native conformation, hence preventing the formation of β_2M fibrils and amyloid activity. Further analysis can be considered, such as studying the effect of varying β_2M concentrations on the mean differential deflection profiles for biosensing purposes. Therefore, this method opens the possibility of designing a mechanical-based immuno-sensor that differs from the previously discussed other types of biosensors.

5. CHALLENGES AND FUTURE TRENDS IN THE DEVELOPMENT OF β_2M BIOSENSORS

The performance of β_2M under biological conditions is affected by many complexes, such as MHC-1 and nonclassical MHC-1-like molecules.[3] Interferences in the binding of β_2M to MHC-1 and nonclassical MHC-1 molecules in the complex medium of biological samples, such as serum and urine, can produce false positive signals and interpretations. Though novel contributions have been made toward developing device-based biosensing platforms, real physiological samples containing β_2M have not been used to evaluate the sensor devices. Therefore, it is important to establish the intended clinical need prior to biosensor development. For instance, multiple biomarkers need to be detected in a clinical sample within specific cutoff values in order to diagnose complex diseases, such as cancer,[37] inflammatory disorders,[68] peripheral arterial disease,[165] and chronic kidney disease,[166,167] that relate to β_2M. This is one of the most significant challenges facing the development of a diagnostic tool without signal overlap because specific working mechanisms that can identify and translate the particular biophysical properties of the target biomarker need to be developed. These devices need to identify multiple rather than individual biomarkers as well as distinguish between varying levels of these biomarkers rather than simply a minimal concentration to zero. As such, the sensitivity, dynamic ranges, and resolutions of these devices are

https://doi.org/10.1021/acsbiomaterials.2c00036
ACS Biomater. Sci. Eng. 2022, 8, 3142−3161

critical parameters that need to be addressed for particular clinical applications. Therefore, detection strategies that use rapid, simple, and accurate methods that are based on established optical and electrochemical detection should be developed and optimized while taking into account the complexity of the biomarkers, the affinity of the biorecognition elements, the clinical cutoff values, the relevant dynamic range, and the sample matrix.

Despite an overwhelming clinical need, the translation of biosensor devices from laboratories to clinical applications has to enjoy limited but notable progresses due to high background or noise signals as well as limited detection accuracy in clinical samples. As such, researchers have dedicated themselves to developing nanomaterial-based immuno-sensors, with many electrode surface modifications, that enhance biorecognition activities for highly sensitive biomarker detection and increased measurement accuracy.[168] Although downscaling sensing materials into two-dimensional (2D), one-dimensional (1D), or zero-dimensional (0D) structures have been found to increase protein and DNA detection down to attomolar and femtomolar ranges,[169,170] the high fabrication cost of the scaled-up productions as well as variations between devices poses a challenge. If the biomarkers in the biosensor array consist of different structures or physical properties, a different set of dynamic ranges could be used to simultaneously detect multiple biomarkers, for instance if a biomarker consists of nucleic acid sequences, proteins, and cells. Furthermore, advanced signal processing integrated with advanced machine learning algorithms, such as convolutional neural networks (CNN), recurrent neural networks (RNN), *k*-nearest neighbor (*k*-NN), and support vector machine (SVM), can be incorporated to improve detection performance (e.g., specificity, sensitivity, and precision) in high-throughput biosensor arrays for β_2M biomarker analysis, which may become a trend as intelligent biosensors in the near future.[171] This, as well as advances in materials, device engineering, and signal processing technologies, indicates that the trend of β_2M biosensor development is evolving toward miniaturization, multiplexing, and wireless networking for data transfer and storage to establish point-of-care testing (POCT) platforms.

6. CONCLUSIONS

In conclusion, β_2M can be used to assess glomerular and tubular function in adults. Of all of the body fluids available, the sampling of serum, lacrimal, and urinary β_2M is adaptable and cost-effective as well as provides the best screening samples for various pre- and postclinical studies. However, the efficacy of using β_2M as a marker in specific case studies is limited because β_2M levels can increase under certain conditions for multiple reasons. Therefore, future studies should examine interactions between β_2M levels in a particular disease and other confounding factors. Unlike other biomarkers, β_2M is a relatively versatile biomarker that can be used for numerous applications. As such, the use of an advanced biosensor platform combined with computer-aided measurement system may improve specificity and the affinity to produce efficient assay schemes.

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P.C.S., K.S.O., and M.A.S.M.H. directed and wrote the main manuscript text. M.A., C.F.D., N.M.M., A.A.H., and E.Y.C. provided support in the review and editing of the manuscript. All authors have read and approved the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was funded by Universiti Kebangsaan Malaysia (UKM), Malaysia under Geran Universiti Penyelidikan (GUP-2021-070). This work was also supported by AKU254: HiCoE "MEMS for Biomedical Devices (Artificial Kidney)" from the Ministry of Education, Malaysia. As part of collaboration, this work was supported by the "Center for the Semiconductor Technology Research" from the Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education, Taiwan. This work was also supported in part by the Ministry of Science and Technology, Taiwan (MOST 111-2634-F-A49-008 and MOST 111-2622-8-A49-018-SB) and National Chung-Shan Institute of Science and Technology (NCSIST-404-V407(111)).

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