生物传感器分析技术及其在阿尔茨海默病药物研发中的研究进展

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摘要: 生物传感器分析技术是一类可将生物反应转换为光电信号的高特异性技术, 在阿尔茨海默病 (Alzheimer's disease, AD) 药物研发中, 针对AD不同假说、不同靶点, 该技术在确证靶标、筛选活性化合物方面发挥了重要作用。该文简述AD发病机制与治疗药物现况, 重点介绍了AD药物研发中常用的表面等离子共振技术 (surface plasmon resonance, SPR)、生物膜干涉技术 (biolayer interferometry, BLI) 与荧光分析技术这三类生物传感器分析技术, 阐释其基本原理及其应用进展, 并总结各自的优势与局限。

关键词: 阿尔茨海默病; 生物传感器; 表面等离子共振技术; 生物膜干涉技术; 荧光分析技术; 药物筛选

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Biosensor analysis technology and its research progress in drug development of Alzheimer's disease

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Abstract: Biosensor analysis technology is a kind of technology with high specificity that can convert biological reactions into optical and electrical signals. In the development of drugs for Alzheimer's disease (AD), according to different disease hypotheses and targets, this technology plays an important role in confirming targets and screening active compounds. This paper briefly describes the pathogenesis of AD and the current situation of therapeutic drugs, introduces three biosensor analysis techniques commonly used in the discovery of AD drugs, such as surface plasmon resonance (SPR), biolayer interferometry (BLI) and fluorescence analysis technology, explains its basic principle and application progress, and summarizes their advantages and limitations respectively.

Key words: Alzheimer's disease; biosensor; surface plasmon resonance; biolayer interferometry; fluorescence analysis technology; drug screening

阿尔茨海默病 (Alzheimer's disease, AD) 作为最常见的神经退行性疾病之一, 其特征是认知功能进行性受损, 包括记忆力、语言、执行功能衰退和社交能力的丧失[1], 在全球范围内约有5 000万人患病[2], 伴随老龄化进程, 其患病人数还在不断上升, 预计到2050年AD患者将增加至1.5亿[3,4]。鉴于现有AD治疗药物数量较少, 疗效较差, 研发更为有效的AD药物迫在眉睫。

生物传感器是一种可以识别特定生物元件的装置, 它能与样品中的物质发生相互作用, 引起物理化学性质变化并转化为可测量的信号[5]。在AD药物研发领域, 生物传感器发挥了重要的作用[6], 生物传感器可以利用针对特定药物靶点设计, 评价小分子与蛋白质、蛋白质−蛋白质相互作用 (protein-protein interaction, PPI) 等, 加速了对潜在候选药物的疗效和毒性的评估,

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并能够针对设计的靶点进行药物筛选。本文着重介绍应用于AD药物研发的生物传感器分析技术, 包括表面等离子共振 (surface plasmon resonance, SPR) 技术、生物膜干涉 (biolayer interferometry, BLI) 技术与荧光分析技术, 综述其基本原理与研究进展, 为AD药物筛选与研发提供技术支撑。

1 阿尔茨海默病的发病机制及治疗药物概述

AD发病机制复杂, 目前存在多种假说, 其中比较受认可的是β淀粉样蛋白 (amyloid-β, Aβ) 沉积与Tau蛋白异常磷酸化假说。Aβ在大脑皮质的沉积是AD患者最常见的病理现象, 生理状态下淀粉样前体蛋白 (amyloid precursor protein, APP) 被α和γ分泌酶消化产生可溶性多肽, 而当β分泌酶与γ分泌酶协同作用时, 不溶性的Aβ产生并聚集, 形成β淀粉样斑块[7], 这些斑块的存在破坏神经元之间的信号传导[8]并诱发神经炎症与免疫反应[9]。而作为微管相关蛋白之一的Tau是组成神经元细胞的骨架, Tau蛋白的异常磷酸化会形成神经原纤维缠结, 导致神经元损伤[10]。其余AD发病机制报道有胆碱能假说[11], 肠道菌群[12,13], APP、早老素1和早老素2[14]及载脂蛋白4[15]基因突变与包括病毒、真菌和细菌在内的系统性感染[16,17]等。

因其病理成因仍未明晰, 目前临床上针对AD的治疗以缓解症状为主, 相关治疗药物[18]包括乙酰胆碱酯酶 (acetylcholinesterase, AChE) 抑制剂氢溴酸加兰他敏、盐酸多奈哌齐和酒石酸卡巴拉汀, 用于提升乙酰胆碱 (acetylcholine, ACh) 水平来改善进行性认知功能障碍[19], 以及*N*-甲基-*D*-天冬氨酸受体拮抗剂盐酸美金刚, 该药物可防止过量谷氨酸过度刺激神经元而引起的兴奋性毒性损伤[20], 但这些药物活性有限, 不能治愈或逆转AD进程[21], 并且胃肠道、神经系统、肌肉痉挛疼痛等不良反应发生率高[22]。此外, 近三年来, 针对Aβ的阿杜那单抗[23] (aducanumab)、仑卡奈单抗[24] (lecanemab) 和靶向脑–肠轴的药物甘露特钠胶囊[25] (GV-971) 分别被FDA与CFDA批准用于AD治疗, 但这些药物同样面临药效或不良反应的争议[26,27]。AD药物研发面临投入大、产出低的困境, 当前药物研发针对不同的假说以及相关靶点多线进行[28]。

中药作为发现天然产物与先导化合物的重要宝库, 具有高度的结构多样性与结构复杂性, 其中经典药物与方剂历经千百年实践验证, 具有巨大的成药潜力[29]。近年来中药在AD药物研发领域受到越来越多的重视[30-33], 已成为除了人源化单克隆抗体、多肽以外的另一大类研究对象。如何从中药中筛选出候选化合物, 进一步探究候选活性化合物的作用靶点等, 生物传感器技术如SPR、BLI以及荧光分析技术等在这些研究领域中发挥着重要作用。

2 生物传感器分析技术及其应用

2.1 表面等离子共振技术

2.1.1 基本原理 SPR技术的发展可追溯到1968年, Kretschmann和Raether[34]通过在高折射率的棱镜底面上镀上几十纳米的金属薄膜, 实现表面等离子共振现象的光学激发, 为今后SPR的研究打下基础。SPR仪器核心为其中的生物传感器芯片, 它通常由光波导耦合器、金属膜 (大多数是金膜) 和具有生物识别功能的敏感膜组成。在药物研发过程中, 作为药物靶标的蛋白被固定在芯片表面, 候选化合物以溶液形式流经芯片表面, 当二者发生相互作用时, 传感器表面质量增加, 引起表面折光角度变化并被软件记录, 通过曲线上共振单位 (resonance unit, RU) 的变化, 得以定性评估化合物是否与靶蛋白结合, 或定量评价二者之间的亲和力, 如图1所示[35]。该技术被广泛用于生物分析[36]、食品检测[37,38]、临床诊断[39-41]以及药物研发[42]等领域, 研究生物分子之间的相互作用, 实现了实时定量测定分子间相互作用力, 计算亲和力与动力学参数等功能, 具有非标记、原位实时、高灵敏度等特点。为避免堵塞流路系统, 溶液需经微孔滤膜或离心后方可进行SPR检测, 对中药提取液这类混合组分而言, 会导致一些成分损失。

随着商品化SPR技术的逐渐成熟, 不同种类的商用芯片应运而生, 研究者可以根据配体特性, 选择合适芯片用以连接, 并调整偶联试剂与时间; 此外, 为满足特定检测需求, 研究人员可以自行修饰裸金传感器, 改良芯片表面结构, 进一步提高其专属性与灵敏度。Wang等[43]利用双金纳米粒子与金膜之间的电子耦合效应辅助放大信号, 用11-巯基-1-十一醇封闭芯片表面, 实现了低浓度外泌体的检测, 检测限低至5×10^3个外泌体·mL^{-1}, 比ELISA提高了1×10^4倍。Xue等[44]开发了一种基于锑烯二维纳米材料的SPR传感器, 对生物标志物miRNA检测限可达1×10^{-17} mol·L^{-1}, 比现有miRNA传感器高2.3～10 000倍, 有望应用于癌症的早期诊断和监测。Tan等[45]在裸金传感器表面修饰5 nm厚度的二维二硫化钼 (MoS_2), 构建了MoS_2-增强型SPR生物传感器, 其磺胺检测限为0.05 μg·L^{-1}, 比裸金SPR系统显著降低。

2.1.2 在AD药物筛选中的应用 借助SPR无需标记、实时快速检测、用量少的特点, 该技术可被用于AD候选药物的高通量筛选与靶标验证研究。SPR技术应用于药物筛选的核心在于确定研究靶点, 即目标蛋白, 并构建稳定捕获该蛋白的传感器芯片。β-分泌酶1 (beta-secretase 1, BACE1) 是一类天冬氨酸型蛋白

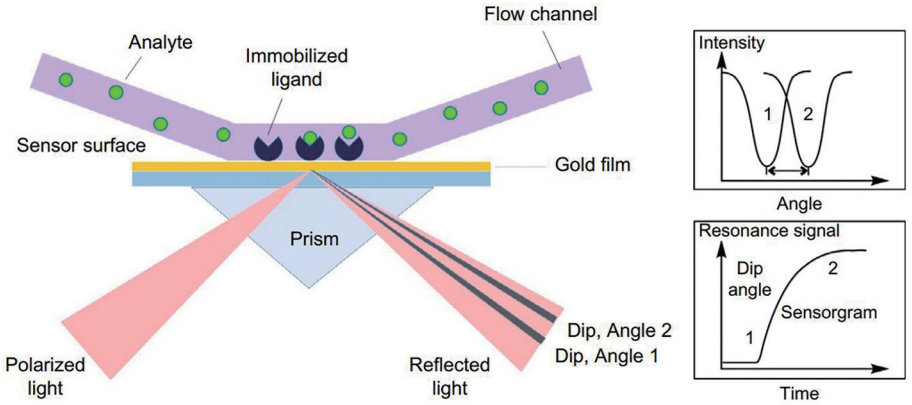

Figure 1 Surface plasmon resonance (SPR) mechanism (reprinted with permission from reference[35], copyright 2018, Elsevier)

酶, 负责APP的初始切割, 随后c-分泌酶复合物对其进一步处理产生Aβ肽, 如$Aβ_{1-40}$、$Aβ_{1-42}$。Christopeit等[46]构建了基于SPR的膜包埋全长BACE1的检测方法。首先活化L1芯片, 通过芯片表面葡聚糖羧基固定有抗组氨酸标签的单克隆抗体 (monoclonal anti-polyhistidine antibody, MAHisA), 偶联量为8 000 RU, 然后借助His6标签从Sf9细胞裂解液中直接捕获包括跨膜结构域的完整BACE1, 同时注射总脑脂提取物维持其在天然脂膜中的构象, 以稳定捕获的蛋白质, 最终该方案固定了1 700～2 200 RU的BACE1, 相比无脂质稳定方案, BACE1-MAHisA-L1芯片稳定性大幅提升至24 h。该芯片中的BACE1更接近生理条件下的蛋白状态, 同时其灵敏度满足与小分子的相互作用研究, 有助于推动该靶点的药物研发。

小胶质细胞的激活是AD主要病理现象之一, Ig超家族的细胞表面受体TREM2 (triggering receptor expressed on myeloid cells 2) 参与这一过程, 抑制神经炎症反应, 同时TREM2的功能缺失可导致患AD风险增加。Wang等[47]建立了以TREM2蛋白为靶点的SPR-UPLC-MS/MS药物筛选系统, 优化了蛋白偶联条件, TREM2用pH 4.0的醋酸钠稀释至20 $mg·mL^{-1}$, 与CM5传感器芯片偶联时间为120 s, 流速为20 $μL·mL^{-1}$, 最终TREM2的偶联量为5 000 RU。向该系统中注入曼陀罗种子提取液, 通过反复结合和解离, 合并回收与传感器表面TREM2蛋白结合的成分, 浓缩后进样分析。应用所建立的SPR-UPLC-MS/MS方法在曼陀罗种子提取液中鉴定出大麻素G、大麻素K、大麻素F与大麻素D, 并采用LPS诱导的小胶质细胞炎症细胞模型研究其抗神经炎症活性机制。

Aβ聚集并导致沉积是AD最显著的特征之一, 金纳米颗粒 (gold nanoparticles, GNPs) 可吸附并介导Aβ快速聚集, 结合其表面等离子体的性质, 在Aβ聚集抑制剂筛选中有很大的应用潜力。Lee等[48]利用较长的纤维状Aβ-GNP聚集体会产生SPR红移, 而Aβ聚集抑制剂则阻止Aβ-GNP聚集, 诱导较短聚集体的形成并表现出SPR峰蓝移的原理, 建立了两步SPR分析法, 如图2所示, 当GNP与Aβ肽聚集时, 其520 nm处的主峰峰强降低, 当Aβ-GNP聚集体长度变长时, 其次级SPR峰发生连续红移, 因此Aβ-GNP聚集体的SPR偏移可以作为Aβ聚集抑制剂抑制活性的指标; 而设计的两步分析法, 分步反应且在不同步骤使用不同pH条件, 可以减少GNP-Aβ抑制剂相互作用。第一步, 保持与生理条件相同的中性pH值, Aβ和聚集抑制剂孵育10 min, 形成Aβ抑制剂复合物; 第二步, 酸性pH值下 (pH 3.2) 加入GNP孵育5 min, 介导Aβ聚合, 监测SPR峰的移动。分别考察了GNP-Aβ聚集抑制剂对SPR峰干扰、有机溶剂及其浓度、pH值、孵育时间等因素的影响, 建立了基于SPR峰偏移的Aβ聚集抑制剂筛选方法, 并使用桑色素、儿茶素、芦丁、姜黄素等文献已报道的活性化合物予以验证, 该方法可在15 min内快速筛选潜在Aβ聚集抑制剂。

此外, 在药物研发过程中, SPR作为一种可以实时测定生物分子间结合和相互作用信号的分析技术, 被广泛应用于验证和计算候选化合物与配体蛋白之间的

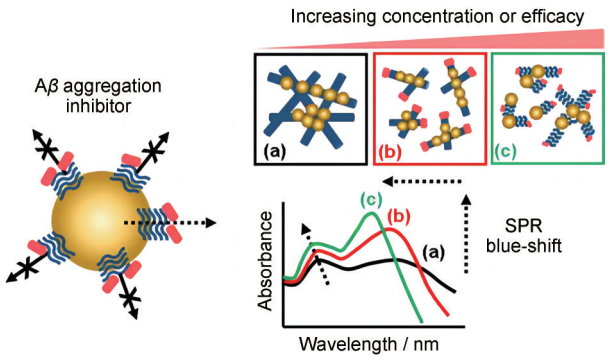

Figure 2 Schematic illustration of the SPR assay for screening aggregation inhibitors (reprinted with permission from reference[48], copyright 2021, American Chemical Society)

亲和力。基于AD最为经典的胆碱能假说, Patil等[49]用SPR技术对已报道有抑制AChE活性的生物碱、多酚等多种天然产物进行评估, 定量其与AChE之间的相互作用, 并进行了竞争性分析物研究。经过偶联条件考察, AChE用pH 4.5的醋酸钠缓冲液溶解, 并固定到CM5传感器芯片上, 固定7 min, 再用乙醇胺 (pH 8.5) 封闭7 min, AChE的偶联量为7 732 RU。分别研究了部分底物–抑制剂和抑制剂–抑制剂之间的相互作用, 保持恒定流速为45 μL·min^{-1}, 结合和解离时间都为120 s。结果表明, 咖啡因 (1.738×10^{-5} mol·L^{-1}) 对AChE的亲和力高于氢溴酸加兰他敏 (1.039×10^{-3} mol·L^{-1}), 多酚类化合物单宁酸 (2.250×10^{-8} mol·L^{-1}) 表现出较高的亲和力。在底物/底物类似物–抑制剂研究中平衡解离常数 (K_D) 值与个体相比有相当大的变化, 而在抑制剂–抑制剂研究中, 藏红花苷和单宁酸较之前的K_D值都增大, 分别为6.022×10^{-3}和1.162×10^{-7} mol·L^{-1}, 其与配体的亲和力降低, 可能原因是两种抑制剂相互竞争结合酶。表1[50-77]列举了自2018年以来SPR技术在AD药物研发过程中的相关应用实例。

2.2 生物膜干涉技术

2.2.1 基本原理 BLI技术是一种通过检测干涉光谱的位移变化来检测传感器表面反应的无标记生物分析方法[78]。如图3所示, 首先仪器经由光纤向传感器发射可见光, 该光在传感器末端光学膜层的两个界面–固定化蛋白质层和内部参考层[79], 会形成两束反射光谱与一束干涉光谱。当固定在生物传感器尖端表面的配体与溶液中的分析物之间发生结合, 生物膜的厚度及密度产生变化, 干涉光谱的波长随之位移[80,81], 从而实现待测分子间相互作用过程的定量测定。基于BLI的系统不需要微流控系统[82], 通常也不需要专用的参考通道, 管路不易发生堵塞, 可检测粗制样品混合物, 如中药提取液等[83]。此外, BLI检测是通过生物传感器尖端表面发生相互作用而产生响应, 因此样品中未结合蛋白质或化合物的变化, 对信号的影响很小, 前处理更简单, 可以保持样品完整性; 而且BLI可以实时监测并计算得到结合速率常数 (k_{on})、解离速率常数 (k_{off}) 和K_D, 而不需要将荧光基团、酶或其他信号元件偶联到蛋白[84], 操作更为便捷快速。BLI技术具有无需标记、实时监测的优点, 但相比于SPR技术, BLI生物传感器的灵敏度较低, 试剂消耗量较大[85]。

为进一步拓展BLI技术的应用范围, 降低干扰信号并提高检测灵敏度, 针对青光眼相关生物标志物生长分化因子-15, Gao等[86]筛选出适配体APT2TM, 并将酶联适配体夹心测定 (enzyme-linked aptamer sandwich assay, ELASA) 集成到BLI系统中, 开发了一个实时自动化, 高通量并具有高灵敏度的BLI-ELASA平台。该平台具有低至5～6 pg·mL^{-1}的检测限和较宽的线性检测范围 (10～810 pg·mL^{-1}), 有望助力青光眼的早期诊断。Hao等[84]开发了一种非标记BLI方法, 用于实时监测恢复期COVID-19患者中受体结合域与SARS-CoV-2刺突蛋白的结合动力学, 该方法不使用任何离液剂, 而是通过使用涂有链霉亲和素多糖的复合生物传感器探针和参比探针来最大限度地减少血清基质中的非特异性结合, 使得干扰降低为原来的50%, 并使信号强度增加6倍。

2.2.2 在AD药物研发中的应用 近年来, 借助其浸入即读的特点, BLI被应用于小分子化合物的筛选与鉴定。西南医科大学吴安国团队[87]应用BLI技术结合UHPLC-DAD-Q/TOF-MS/MS技术, 建立了从中药筛选潜在Aβ聚集抑制剂的方法。首先将靶蛋白A$\beta_{1\text{-}42}$生物素化, 借助链霉亲和素–生物素反应与BLI链霉亲和素传感器相连接, 与中药提取液结合、解离各120 s, 反复解离5次以尽可能收集有效成分, 最终从中药复方开心散提取液中鉴定出猪苓酸C、去氢土莫酸和土莫酸三个候选化合物, 经验证, 这三个化合物可显著抑制Aβ纤维形成, 提高A$\beta_{1\text{-}42}$处理的PC-12细胞的活力, 并改善AD秀丽隐杆线虫的行为能力。该团队在此之前[88]对于黄芩提取液中与A$\beta_{1\text{-}42}$共孵育后筛选得到的化合物–黄芩苷和黄芩素, 用连有A$\beta_{1\text{-}42}$超级链霉亲和素BLI传感器验证这两个化合物的亲和力。结果表明, 黄芩苷和黄芩素的K_D值分别为2.42×10^{-4}和1.7×10^{-4} mol·L^{-1}, 表明黄芩苷和黄芩素与A$\beta_{1\text{-}42}$之间存在相互作用。

此外, BLI技术被广泛用于测量抗原–抗体[89,90]、蛋白–蛋白[91,92]、小分子–配体[93-95]之间的相互作用。Zhao等[96]设计了一种TREM2双特异性抗体Ab2 TVD-Ig, 将二价IgG化合物改造为四价TVD-Ig, 双特异性体现在既为TREM2激动剂, 又同时靶向转铁蛋白受体 (transferrin receptor, TfR) 以透过血脑屏障, 从而使该抗体的入脑浓度增加10倍以上。为验证其作用靶点, TREM2首先同样借助链霉亲和素–生物素反应固定在生物传感器上, 通过先结合抗体, 再结合TfR的BLI实验, 组成“TREM2+Ab2 TVD-Ig/αTfR+TfR”夹心, 借助结合时响应曲线上升, 判断Ab2 TVD-Ig/αTfR双特异性抗体是否可以和TREM2与TfR相互作用。He等[97]研究商陆皂苷甲 (esculentoside A, EsA) 缓解三重转基因AD小鼠认知缺陷的潜在机制, 免疫荧光和蛋白质印迹方法测得过氧化物酶体增殖物激活受体γ (peroxisome proliferators-activated receptor γ, PPARγ) 表达上调, BLI实验采用生物素化的PPARγ与传感器固定15 min后, 进样不同浓度EsA (3.125～50 μmol·L^{-1}), 其K_D值为

Table 1 Applications of SPR in Alzheimer's disease (AD) drug development

Ligand	Source	Receptor	Protein coupling capacity	K_D/mol·L^{-1}	Biosensor	Reference
Aaptamine	*Aaptos suberitoides*	AChE	7 000 RU	8.76×10^{-5}	CM5	[50]
	Brøndsted	BuChE	6 600 RU	1.07×10^{-5}		
SXkmer-YLTIRLM	Phage-display libraries	Aβ(M1-42)	12 000 RU		CM3	[51]
Caffeine		Notum	2 500 RU	8.5×10^{-5}	SA	[52]
Dendritic polyglycerol sulfates	Nanoparticles	A$\beta_{1\text{-}40}$			SIA Kit Au	[53]
		A$\beta_{1\text{-}42}$				
hPT3	Monoclonal antibody	PHF-Tau	6 500 RU		CM5	[54]
MG-2119	Chemical libraries	Tau		$(1.34\pm0.35)\times10^{-6}$	CM5	[55]
Ginnalin A	*Acer rubrum*	A$\beta_{1\text{-}42}$		$(3.5\pm1.1)\times10^{-6}$	CM5	[56]
Ibuprofen/human serum albumin		A$\beta_{1\text{-}40}$	6 000−9 000 RU		GLH	[57]
		A$\beta_{1\text{-}42}$				
		A$\beta_{1\text{-}40}$(A21G)				
Timosaponin A-III	Rhizoma Anemarrhenae	BACE1		1.739×10^{-5}	CM5	[58]
Astaxanthin		A$\beta_{1\text{-}42}$	5 ng A$\beta_{1\text{-}42}$ per 1 mm^2		Gold-coated chips	[59]
β-Boswellic acid		Tau		8.45×10^{-7}	CMD	[60]
Lecanemab	Monoclonal antibody	Various forms of Aβ			CM5	[61]
Aducanumab						
Gantenerumab						
9 Compounds	*Pancratium* L.	AChE			CM5	[62]
		A$\beta_{1\text{-}42}$				
Hexa-RmAb158	Monoclonal antibody	A$\beta_{1\text{-}40}$			CM5	[63]
		A$\beta_{1\text{-}42}$				
Sanguinarine	Isoquinoline alkaloids	A$\beta_{1\text{-}42}$		4.63×10^{-4}	CM5	[64]
Chelerythrine				3.83×10^{-3}		
Coralyne				1.16×10^{-5}		
Purpurin		FL-Tau			GLC	[65]
Donepezil-loaded apolipoprotein		A$\beta_{1\text{-}42}$ monomer		2.45×10^{-8}		[66]
A-I-reconstituted HDL (rHDL/Do)		A$\beta_{1\text{-}42}$ oligomer		2.78×10^{-8}		
ANK3	Lead compound	A$\beta_{1\text{-}42}$	1 500−1 800 RU	7×10^{-6}	SA	[67]
ANK6	Denantiomeric			3×10^{-6}		
Salidroside		NRF2				[68]
Serotonin/human serum albumin		A$\beta_{1\text{-}40}$		6×10^{-8}	GLH	[69]
		A$\beta_{1\text{-}42}$		1.2×10^{-7}		
A9	DTP library	Biotinylated heparin	150 RU	$(1.1\pm0.8)\times10^{-5}$	SA	[70]
1,2,3,4-Tetrahydro-1-acridone analogues 30	Synthesis	A$\beta_{1\text{-}42}$		1.60×10^{-5}	GLH	[71]
		Tau		3.37×10^{-5}		
K11	Monoclonal antibody	Various forms of Aβ		(2−2 700)$\times10^{-9}$	CM5	[72]
Tetrapeptide Ac-HAEE-NH_2		A$\beta_{16\text{-}G4\text{-}C}$	1 023 RU	$(9\pm3)\times10^{-5}$	CM5	[73]
Cyanobacterial protein phycoerythrin	Cyanobacteria	BACE1	4 500 RU	$(1.75\pm0.134)\times10^{-6}$	GLC	[74]
Trilobatin		HMGB1		8.541×10^{-4}	CM5	[75]
Dendrobine	*Dendrobium nobile* Lindl.	CDK5		2.05×10^{-4}	CM5	[76]
Tetrapeptide HAEE	Synthesis	A$\beta_{1\text{-}42}$		$(4.1\pm0.3)\times10^{-6}$	CM4	[77]
		isoD7-Aβ_{42}		$(1.04\pm0.04)\times10^{-5}$		

2.54×10^{-6} mol·L^{-1}, 证实 EsA 可与 PPARγ 结合。Zhou 等[98]合成了一种新型以锌为中心元素的多孔碳, 可稳定包被 Aβ 形成 Aβ@Zn-HPC 用于配体垂钓, 具有高度选择性。利用该原子材料结合 HPLC-LTQ-Orbitrap 质谱, 成功从姜黄中筛选出姜黄素和双去甲氧基姜黄素两个候选化合物, BLI 法在此用于证实固定在生物传感器尖端表面的 Aβ 与这两个化合物存在结合, 表现为传感器层的光学厚度增加, 监测到的干涉波长偏移。

2.3 荧光分析技术

2.3.1 基本原理 在 AD 药物研发过程中, 荧光共振能量转移 (fluorescence resonance energy transfer, FRET) 原理较常被使用, 其基础是一个荧光基团 (供体) 与另一个基团 (受体) 处于共振态时会发生能量传递[99], 即以供体的激发波长激发, 可观察到受体发射荧光。发生 FRET 有两个基本要求: 供体发射光谱和受体吸光光谱之间必须有一定的重叠, 并且这两个分子必须非

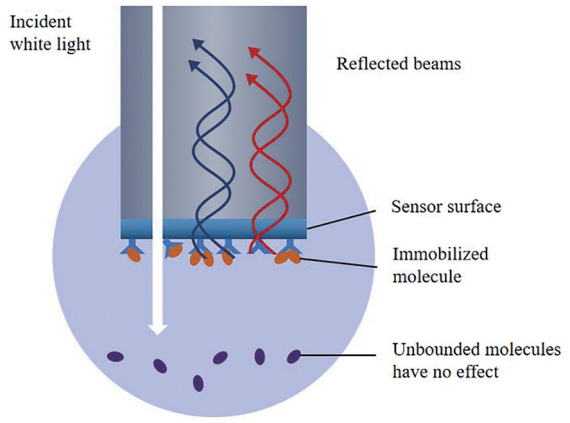

Figure 3 Biolayer interferometry (BLI) mechanism

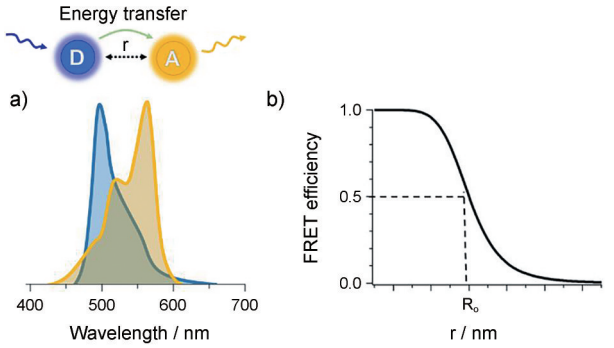

Figure 4 Fluorescence resonance energy transfer (FRET) mechanism (reprinted with permission from reference[100], copyright 2022, John Wiley and Sons). a: Demonstration of a donor (blue) and acceptor (yellow) molecule exhibiting sufficient spectral overlap between their emission and absorbance spectra, respectively, for FRET to occur; b: Relationship of the FRET efficiency of adonor/acceptor pair as a function of distance between the two molecules

常接近 (约 1～10 nm)[100], 如图 4 所示, 而生物体内 PPI 与药物–靶点相互作用正符合这一要求, 因此 FRET 被广泛应用于检测大分子的构象变化或研究与其靶标结合的配体, 相比 SPR 与 BLI 技术, 在测量蛋白质与配体的结合亲和力方面, 该技术所需的蛋白质/配体的数量相对较少, 并且蛋白质或配体都不需要固定在表面上。这使得 FRET 分析完全基于溶液, 并最大程度减少因为蛋白被固定, 使得与配体结合时结构变化可能的限制[101,102], 在活细胞的生理条件下实时动态地研究 PPI, 并具有高灵敏度与特异性, 同时可实现快速检测[103], 但是其缺点在于需要根据待检测的蛋白设计特定荧光标记的分子, 操作更为复杂。

因此, 设计合适的小分子荧光探针是实现荧光分析的关键。总体策略是将彼此非常接近的供体–受体对锚定在一个系统中, 然后通过合成引入特定识别基团以构建最终的 FRET 系统[104]。针对亨廷顿舞蹈症中亨廷顿外显子蛋白 1 (Httex1) 错误折叠并发生病理性聚集, 以及多聚谷氨酰胺扩展突变, Lo 等[105]设计了基于时间分辨-FRET 的生物传感器, 将表达绿色与红色荧光蛋白的基因与 Httex1 基因融合, 并用 Gly-Ser-Leu 连接子连接, 设计出的 FRET 生物传感器可监测不同扩展谷氨酰胺长度 (Q39 和 Q72) 的 Httex 1 在活细胞中的聚集, 已应用于 Httex1 聚集小分子抑制剂的高通量筛选。

2.3.2　在 AD 药物研发中的应用　近期, FRET 原理被用于开发基于喷墨打印的 BACE1 抑制剂筛选方法[106], 通过在羊皮纸的相同位置打印 BACE1 FRET 底物与 BACE1 蛋白, 检测加入样品后该点位的荧光强度, 用于筛选潜在的 BACE1 抑制剂。实验中, 保持底物浓度恒定并增加抑制剂的量, 建立荧光曲线来评价抑制剂药效。这种方法优点在于进行抑制实验所需的酶和底物量非常少。该方法需要的 BACE1 和底物数量不足传统孔板实验的 0.1%, 筛选出的化合物抑制活性实验结果具有良好重现性, 适用于 BACE1 抑制剂的高通量筛选。*O*-连接的 *N*-乙酰葡糖胺水解酶 (*O*-GlcNAcase, OGA) 是生物体内唯一水解蛋白质 *O*-GlcNAc 修饰的糖苷酶, 而乙酰葡糖胺糖基化修饰上调与稳定 Tau 蛋白, 防止其磷酸化并聚集密切相关。Yuzwa 等[107]合成了一种新的荧光探针用于 OGA 抑制剂的筛选, 合成过程快速且产率高, 该探针既适用于人 OGA, 也适用于产气荚膜梭菌的同源细菌 CpOGA, 并验证了其适用于荧光分析, 从结构上表征了 CpOGA 与从片段文库筛选中鉴定出的配体形成的络合物。Lo 等[108]设计了两种新型的细胞 FRET 生物传感器, 在 HEK293 细胞中表达了与绿色荧光蛋白或红色荧光蛋白融合的全长 2N4R 野生型 Tau, 并使用荧光寿命平板阅读器, 相比传统的荧光强度检测, 荧光寿命检测将基于 FRET 筛选的精确度提高了 30 倍, 其高灵敏度使分子间/分子内 Tau 相互作用导致的微小结构变化更易被发现。该平台被用于高通量筛选, 并识别出一种新的工具化合物 MK-886, 它可直接与 Tau 结合, 干扰有毒的 Tau 寡聚体构象, 并减轻 Tau 诱导的细胞毒性, 验证了该筛选平台的可行性。组胺是一种神经递质, 其中组胺 3 受体 (histamine H3 receptor, H3R) 的激活与记忆过程和认知作用有关, 临床试验中, 几种阻断 H3R 的候选药物增加 ACh 的释放[109,110], 减缓 AD、精神分裂症等神经系统疾病的进展。Liu 等[111]构建了一种新型的生物传感器, 可以通过分子内 FRET 信号来可视化 H3R 的激活。将青色荧光蛋白连接在 H3R 的 C-末端, 将黄色荧光蛋白插入第三胞内环, 当配体结合在配体结合位点周围诱导 GPCR 内的小构象变化, 信号被放大并被传递给 G 蛋白。因此,

Table 2 Characteristics of three biosensors

Type	Application	Advantage	Disadvantage
SPR	Affinity determination	① High sensitivity; ② High throughput; ③ Offer binding kinetics.	① High cost; ② Need for proper immobilization strategy; ③ Reference of organic solvent.
BLI	Affinity determination	① High throughput; ② Offer binding kinetics.	① Low sensitivity; ② Requires a minimum sample volume of 80 μL.
Fluorescence	Efficacy assessment; toxicity evaluation	① High sensitivity; ② Low cost; ③ Can be detected in living cells.	① Low efficiency; ② False positives and false negatives; ③ Require synthesis steps; ④ Steric hindrance.

如果FRET供体和受体在GPCR多肽内的适当位置融合, 它们的相对距离或方向可能会发生变化, 从而导致FRET信号发生变化。结果表明, H3R激动剂处理以时间和浓度依赖的方式增加了FRET信号, k_{on}和k_{off}值与已发表的数据一致。同时FRET信号可被H3R拮抗剂抑制, 该FRET传感器可用于配体库筛选、配体结合动力学研究和许多其他药理学分析。

3 小结与展望

在AD药物研发过程中, SPR、BLI和荧光分析技术这三大类生物传感器分析技术被广泛应用于活性化合物筛选、蛋白靶点确证等方面, 各有其适用范围及优缺点。SPR技术是其中首个被2020版中国药典[112]收录的分析技术, 被用于亲和力与动力学测定、结合特异性分析、表位分析和活性浓度检测, 可以实现生物相互作用的实时定量, 灵敏度高, 但构建芯片的过程对维持目标蛋白稳定有要求, 同时蛋白偶联量会随时间推移降低, 并且信号会受溶剂效应的影响, 使用时需要进行校正。BLI技术与SPR应用范围类似, 可提供实时亲和力常数信息, 相比SPR受溶剂影响较小, 但灵敏度较低且试剂消耗量大。基于荧光的分析技术, 优点在于可活细胞中实时进行生化分析, 但荧光需要额外修饰或合成步骤, 并需考虑假阳性和假阴性情况, 同时荧光基团较大, 其空间位阻可能影响蛋白自身功能, 妨碍蛋白与之配体正常结合。表2总结了SPR、BLI和荧光分析技术这三类生物传感器的应用场景以及优缺点。

尽管各有优劣, 生物传感器分析技术还是因其高特异性备受研究者青睐, 依托SPR、BLI和荧光分析技术的各自特点, 针对不同对象, 可设计更加高通量、快速便捷的药物筛选方法, 加速AD药物研究。目前生物传感器研究有以下几个潜在的发展方向: ① 进一步优化SPR、BLI传感器表面设计, 使其与目的蛋白连接更加稳定, 探索通用便捷的偶联方式, 降低传感器的检测限、扩大检测浓度范围; ② 开发新的蛋白稳定策略, 保证配体在生物传感器中的天然构象与活性, 提升药物筛选的准确性; ③ 研发集成式生物传感器平台, 进一步推动药物筛选实现自动化与高通量; ④ 结合不同传感器特征, 多技术策略组合实现筛选的经济高效。伴随国产仪器研发技术进一步提高, 更加物美价廉的分析仪器与更简单的操作步骤有望推动生物传感器分析技术在AD药物研发及其他研究领域更为广泛的应用。

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利益冲突: 本文无利益冲突。

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