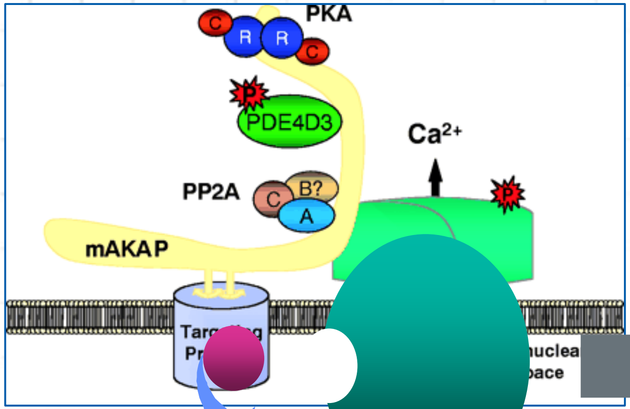
BLI生物膜干涉技术介绍

以及在分子互作研究中的应用

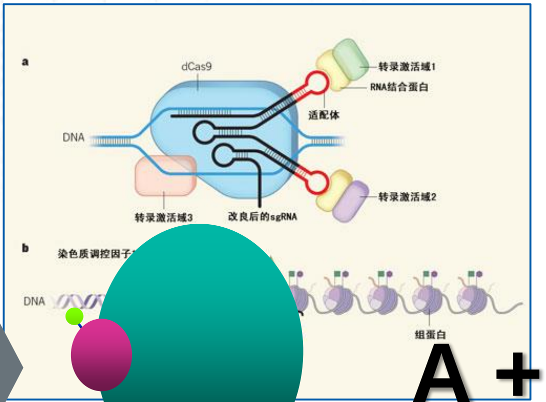
张财辉 13918182485
caihui_zhang@pall.com

为什么要研究分子互作？

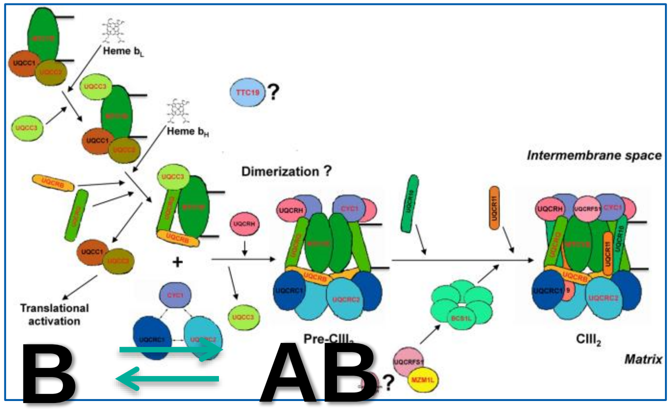
信号传导



基因调控

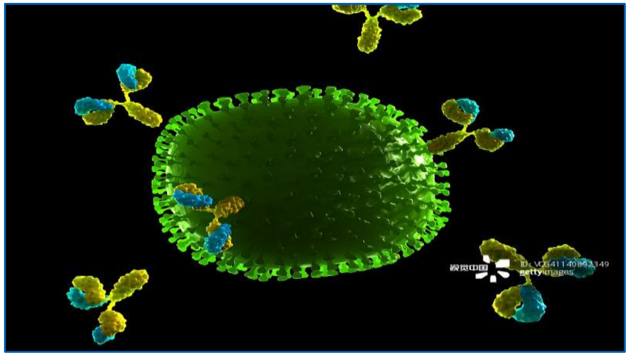


蛋白复合物组装



A + B ⇌ AB

抗体



酶功能

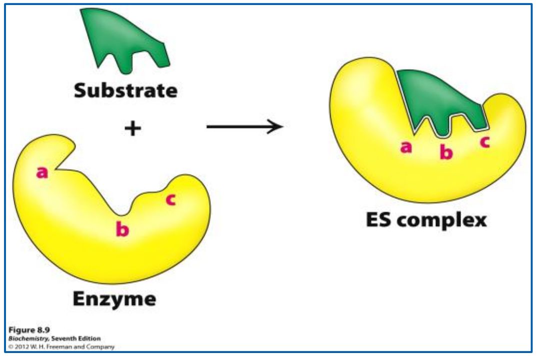
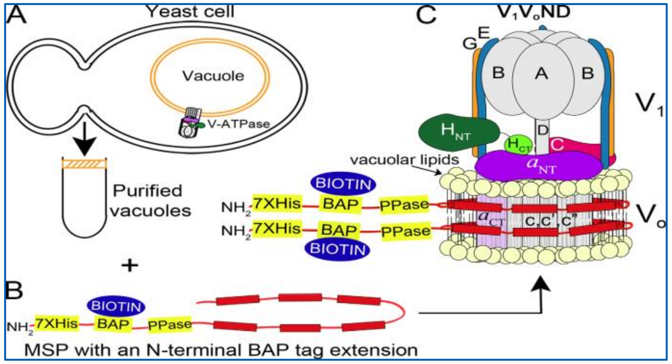


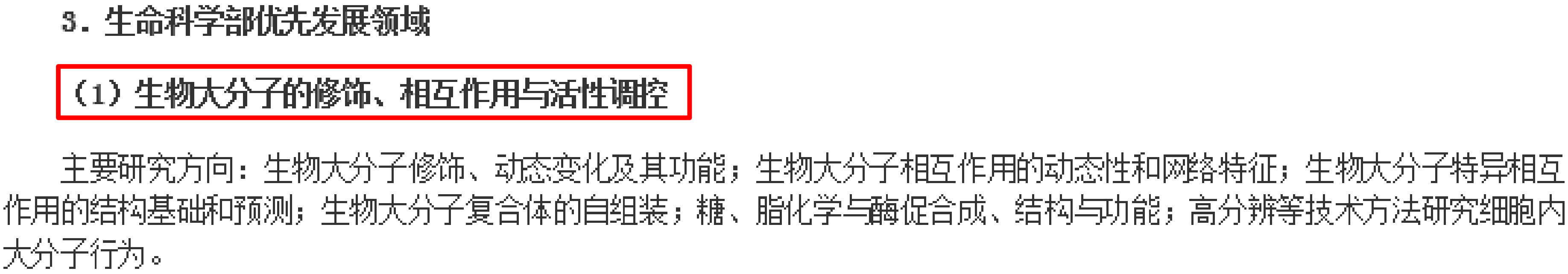
Figure 8.9
Biochemistry, Seventh Edition
© 2012 W. H. Freeman and Company

膜蛋白



生物分子的相互作用


国家自然科学基金委员会
National Natural Science Foundation of China
国家自然科学基金
"十三五" 发展规划


3．生命科学部优先发展领域

（1）生物大分子的修饰、相互作用与活性调控

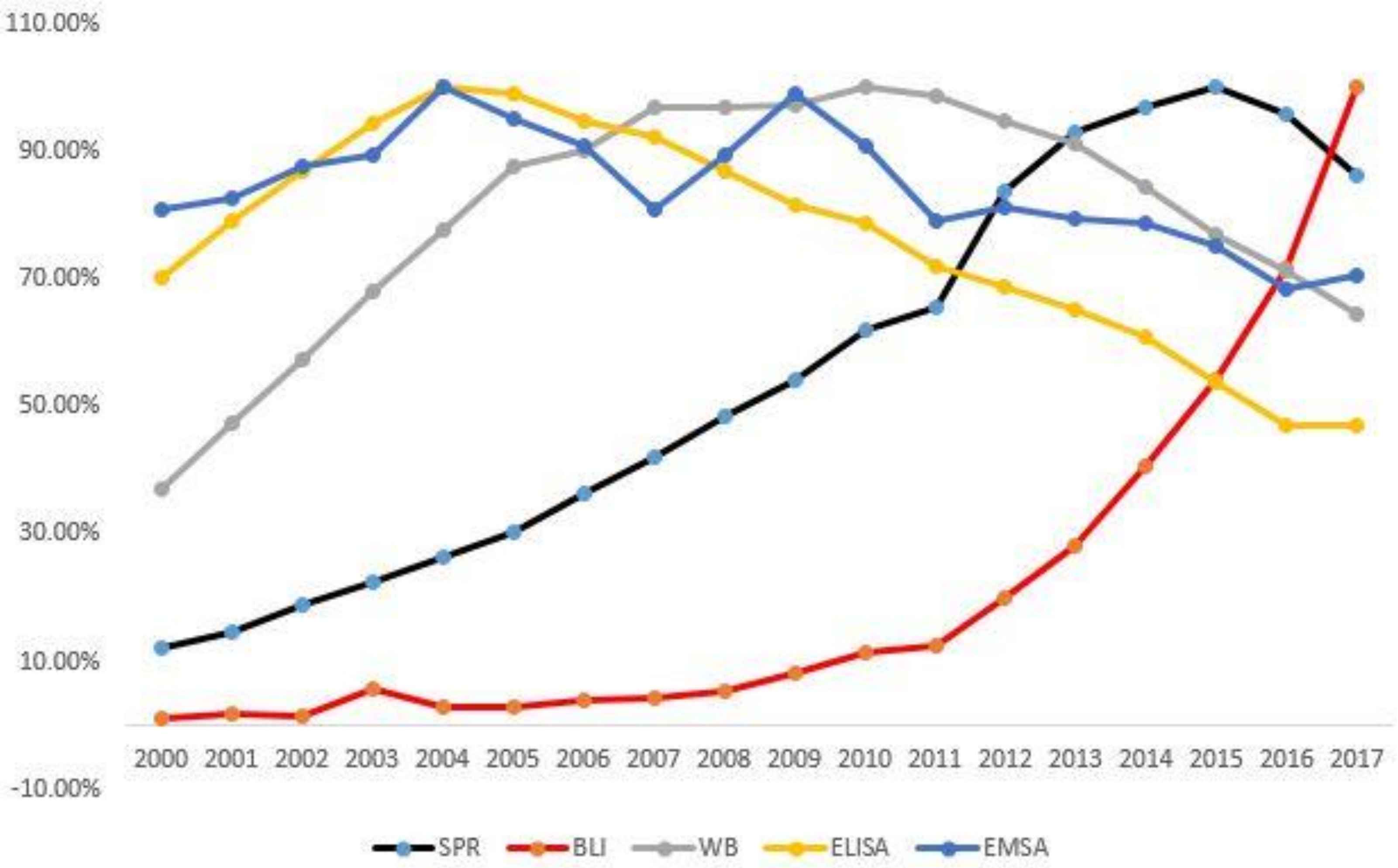
主要研究方向：生物大分子修饰、动态变化及其功能；生物大分子相互作用的动态性和网络特征；生物大分子特异相互作用的结构基础和预测；生物大分子复合体的自组装；糖、脂化学与酶促合成、结构与功能；高分辨等技术方法研究细胞内大分子行为。

研究方法：

传统方法（Label技术）：免疫共沉淀、Pull Down 、EMSA实验、酵母双杂交、ELISA实验、荧光偏振、MST等。

Label Free技术：表面等离子共振 SPR、微量热滴定 ITC、**生物膜干涉 BLI**。

不同技术发表文章增长率对比（google scholar）



内 容

简介

原理

应用

系统特点

公司简介

- ForteBio公司成立于2001年，总部设在美国旧金山，2012年加入Pall公司
- 2015年进入DANAHER集团
- 全球范围装机量超过2000台
- 2009年进入中国市场，安装数量**约200台**
- 目前为**全球最大的分子互作技术厂商之一**

fortéBIO®
A Division of Pall Life Sciences

DANAHER
MOTION

全球用户>2000个，中国>200个

中科院生物物理所
中科院上海生化细胞所
中科院微生物所
中科院遗传发育所
中科院大学生科院
中科院昆明植物所
中科院武汉病毒所
中科院水生所
中科院上海药物所
清华大学
北京大学
上海交通大学
武汉大学
吉林大学
军事医学科学院
复旦大学医学院
中国医科院医生计所

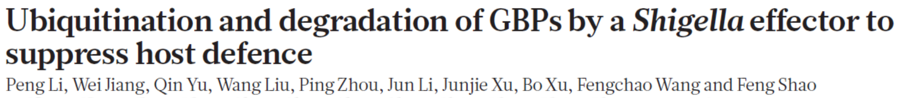
中检院（CFDA）
北京安贞医院
北京儿童医院
北京胸科医院
上海市肺科医院
川大华西医院
山大齐鲁医院
第二军医大学
第三军医大学
第四军医大学
中国医科大学
大连医科大学
中国药科大学
浙江省医科院
昆明医学生物学研究所
广东健康研究院
中医研究院基础研究所

诺和诺德中国研发中心
诺华中国研发中心
百济神州
义翘神州
药明康德
康龙化成
恒瑞医药
信达生物
中信国建
齐鲁制药
金赛药业
睿智化学
中山康方
金凯生物
香雪制药
绿叶制药
康弘药业………

中国用户高水平文献

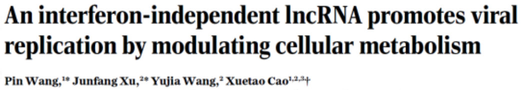
2012至今，中国用户共计发表SCI文献 > 200篇

北京生命科学研究所，邵峰 研究员 2017 Nature


Ubiquitination and degradation of GBPs by a *Shigella* effector to suppress host defence

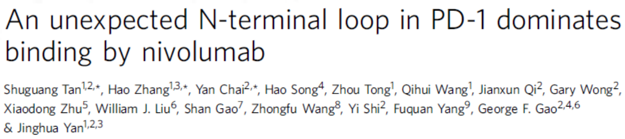
Peng Li, Wei Jiang, Qin Yu, Wang Liu, Ping Zhou, Jun Li, Junjie Xu, Bo Xu, Fengchao Wang and Feng Shao

第二军医大学，曹雪涛 教授 2017 Science

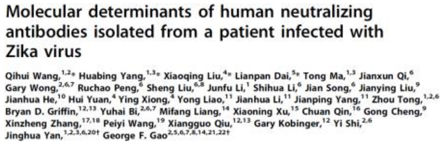

An interferon-independent lncRNA promotes viral replication by modulating cellular metabolism

Pin Wang,[1*] Junfang Xu,[2*] Yujia Wang,[2] Xuetao Cao[1,2,3†]

中科院微生物所，高福 研究员 2016 / 17 Science Translational Medicine / Nature Communications

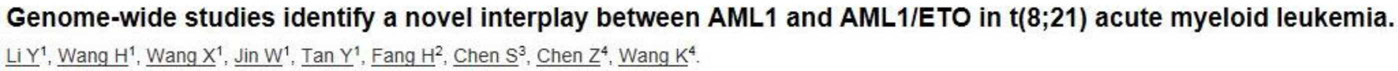

An unexpected N-terminal loop in PD-1 dominates binding by nivolumab

Shuguang Tan[1,2,*], Hao Zhang[1,3,*], Yan Chai[2,*], Hao Song[4], Zhou Tong[1], Qihui Wang[1], Jianxun Qi[2], Gary Wong[2], Xiaodong Zhu[5], William J. Liu[6], Shan Gao[7], Zhongfu Wang[8], Yi Shi[2], Fuquan Yang[9], George F. Gao[2,4,6] & Jinghua Yan[1,2,3]


Molecular determinants of human neutralizing antibodies isolated from a patient infected with Zika virus

Qihui Wang,[1,2*] Huabing Yang,[1,3*] Xiaoqing Liu,[4*] Lianpan Dai,[5*] Tong Ma,[1,3] Jianxun Qi,[6] Gary Wong,[2,6,7] Ruchao Peng,[6] Sheng Liu,[6,8] Junfu Li,[1] Shihua Li,[6] Jian Song,[6] Jianying Liu,[9] Jianhua He,[10] Hui Yuan,[4] Ying Xiong,[4] Yong Liao,[11] Jianhua Li,[11] Jianping Yang,[11] Zhou Tong,[1,2,6] Bryan D. Griffin,[12,13] Yuhai Bi,[2,6,7] Mifang Liang,[14] Xiaoning Xu,[15] Chuan Qin,[16] Gong Cheng,[9] Xinzheng Zhang,[17,18] Peiyi Wang,[19] Xiangguo Qiu,[12,13] Gary Kobinger,[12] Yi Shi,[2,6] Jinghua Yan,[1,2,3,6,20†] George F. Gao[2,5,6,7,8,14,21,22†]

上海交通大学医学院，瑞金医院上海血研所，王侃侃 教授 2016 Blood


Genome-wide studies identify a novel interplay between AML1 and AML1/ETO in t(8;21) acute myeloid leukemia.

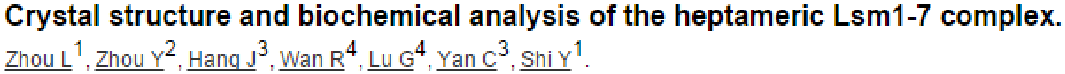
Li Y[1], Wang H[1], Wang X[1], Jin W[1], Tan Y[1], Fang H[2], Chen S[3], Chen Z[4], Wang K[4].

清华大学 李海涛教授 2014 Nature


ZMYND11 links histone H3.3K36me3 to transcription elongation and tumour suppression.

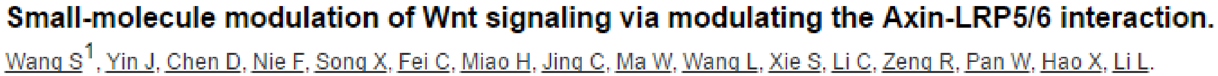
Wen H[1], Li Y[2], Xi Y[3], Jiang S[4], Stratton S[4], Peng D[4], Tanaka K[4], Ren Y[5], Xia Z[6], Wu J[7], Li B[7], Barton MC[8], Li W[6], Li H[5], Shi X[8].

清华大学 施一公教授 2014 Cell Research


Crystal structure and biochemical analysis of the heptameric Lsm1-7 complex.

Zhou L[1], Zhou Y[2], Hang J[3], Wan R[4], Lu G[4], Yan C[3], Shi Y[1].

中科院生化细胞所 李琳研究员 2013 Nature Chemical Biology


Small-molecule modulation of Wnt signaling via modulating the Axin-LRP5/6 interaction.

Wang S[1], Yin J, Chen D, Nie F, Song X, Fei C, Miao H, Jing C, Ma W, Wang L, Xie S, Li C, Zeng R, Pan W, Hao X, Li L.

全球高水平文献众多 CNS>100篇

Nature immunology	2015	T cell receptor dwell times control the kinase activity of Zap70	Salk Institute for Biological
Blood	2015	Structural antidote f	
Nature	2015	Glutathior intracellul	
Nature Medicine	2015	Hemagglu influenza	
Science	2014	A Unique Blocks Ar	
Immunity	2014	Antibody immune p phylogene	
Nature	2014	Structure HIV-1 Env	
Nature	2014	ZMYND1: elongatior	
Blood	2014	A von Wil domains i mice	
Nature	2014	Receptor	
Science	2013	Preferenti influenza	
Science	2013	Structure- for Respir	
Cell	2013	14-3-3 Pr Phosphor	
Nature immunology	2013	Nck-medi the PI(3)K	
Nature Chemical Biology	2013	Small-mo the Axin-L	
Nature	2013	Receptor influenza	
Nature	2013	Coagulati by natura	
Nature	2013	Receptor	
Nature	2013	Conforma endosom	
Science	2012	Synthetic evolution	
Blood	2012	Self-antig receptors	

Nature structural & molecular biology	2016	Neddylatic activated I
Nature	2016	Molecular egg JUNC
Nature	2016	Continuou overcome
Science	2016	Isolation c the 2014 E
Science	2016	Structural by protect
Science	2016	Protective by a poter
Nature Biotechnology	2016	Comprehe Inhibitor S
Nature Biotechnology	2016	Enhancing catalyticall asymmetri
Cell	2016	Cross-Rea Response Infection
Science	2015	Pharmaco Restores
Science	2015	A stable tr protective
Nature	2015	Phosphory inflammati
Nature Immunology	2015	PARP9-DT and viral 3 control vir
Blood	2015	Genome-w AML1 and
Cell	2015	Viral Rece Germline
Nature structural & molecular biology	2015	Crystal str interaction
Cell	2015	Rational D the Recep
Cell	2015	Mechanisı Marburg V
Nature structural & molecular biology	2015	Structure insight int
Nature	2015	Receptor- bacterial i
Nature	2015	HSP70 se erythropoi
Nature	2015	Therapeut castration-
Immunity	2015	Structure Lectin Rec Presentati

Nature Chemical Biology	2017	Recognition of EGF-like domains by the Notch-modifying O-fucosyltransferase POFUT 1	University of Toronto
Immunity	2017	HLA-F: A New Kid Licensed for Peptide Presentation	National Institute of Allergy and Infectious Diseases
Nature Biotechnology	2017	Computational design of trimeric influenza-neutralizing proteins targeting the hemagglutinin receptor binding site	University of Washington
Immunity	2017	Virus-like Particles Identify an HIV V1V2 Apex-Binding Neutralizing Antibody that Lacks a Protruding Loop	National Institutes of Health
cell	2017	Immunization-Elicited Broadly Protective Antibody Reveals Ebolavirus Fusion Loop as a Site of Vulnerability	University of Maryland
Immunity	2017	Glycine Substitution at Helix-to-Coil Transitions Facilitates the Structural Determination of a Stabilized Subtype C HIV Envelope Glycoprotein	The Scripps Research Institute
Nature	2017	Hypersensitive termination of the hypoxic response by a disordered protein switch	The Scripps Research Institute
Immunity	2017	Particulate Array of Well-Ordered HIV Clade C Env Trimers Elicits Neutralizing Antibodies that Display a Unique V2 Cap Approach	Tumor and Cell Biology, Karolinska Institutet
Cell	2017	Antibodies from a Human Survivor Define Sites of Vulnerability for Broad Protection against Ebolaviruses	Albert Einstein College of Medicine
Science	2016	CryoEM structure of a native, fully glycosylated and cleaved HIV-1 envelope trimer	The Scripps Research Institute
Nature Medicine	2016	Influenza immunization elicits antibodies specific for an egg-adapted vaccine strain	Harvard Medical School
Cell	2016	Mitochondrial Sirtuin Network Reveals Dynamic SIRT3-Dependent Deacetylation in Response to Membrane Depolarization	Harvard Medical School
Cell	2016	Cross-Neutralizing and Protective Human Antibody Specificities to Poxvirus Infections	Vanderbilt Vaccine Center
Cell	2016	Induction of HIV Neutralizing Antibody Lineages in Mice with Diverse Precursor Repertoires	Boston Children’s Hospital
Nature structural & molecular biology	2016	Iterative structure-based improvement of a fusion-glycoprotein vaccine against RSV	Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health
Nature	2016	The structural basis of modified nucleosome recognition by 53BP1	The Lunenfeld-Tanenbaum Research Institute
Cell	2016	Structural Basis of Zika Virus-Specific Antibody Protection	Washington University School of Medicine
Blood	2016	Prothrombotic Skeletal Muscle Myosin Directly Enhances Prothrombin Activation by Binding Factors Xa and Va	The Scripps Research Institute
Nature structural & molecular biology	2016	Neddylation requires glycyl-tRNA synthetase to protect activated E2	The Scripps Research Institute
Nature	2016	Molecular architecture of the human sperm IZUMO1 and egg JUNO fertilization complex	University of Toronto
Nature	2016	Continuous evolution of Bacillus thuringiensis toxins overcomes insect resistance	Harvard University
Science	2016	Isolation of potent neutralizing antibodies from a survivor of the 2014 Ebola virus outbreak	The Scripps Research Institute
Science	2016	Structural and molecular basis for Ebola virus neutralization by protective human antibodies	National Institutes of Health
Science	2016	Protective monotherapy against lethal Ebola virus infection by a potently neutralizing antibody	Institute for Research in Biomedicine

内容

- 简介
- 原理
- 应用
- 系统特点

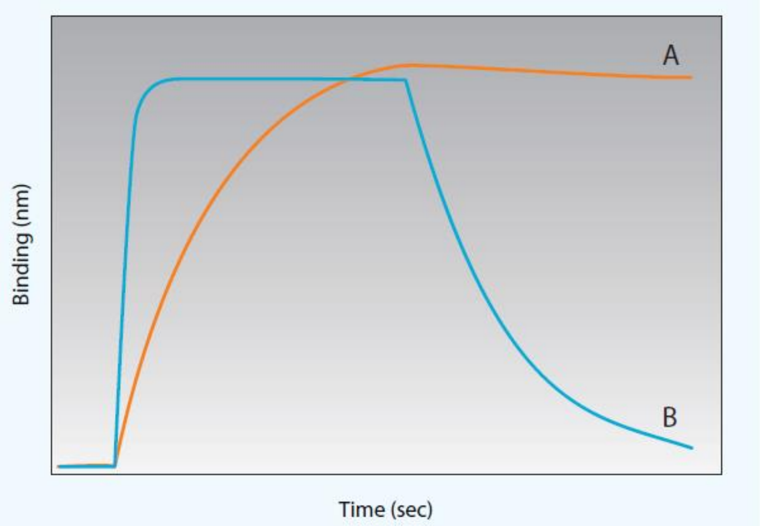
Octet平台介绍

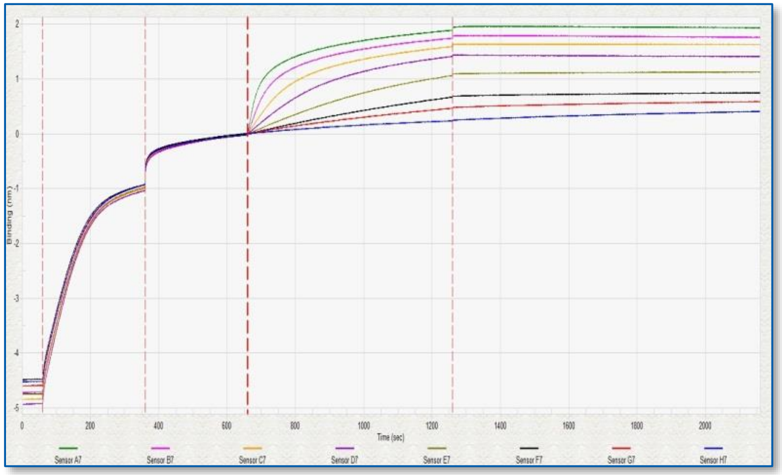
生物膜干涉技术

（Biolayer-interferometry , BLI）

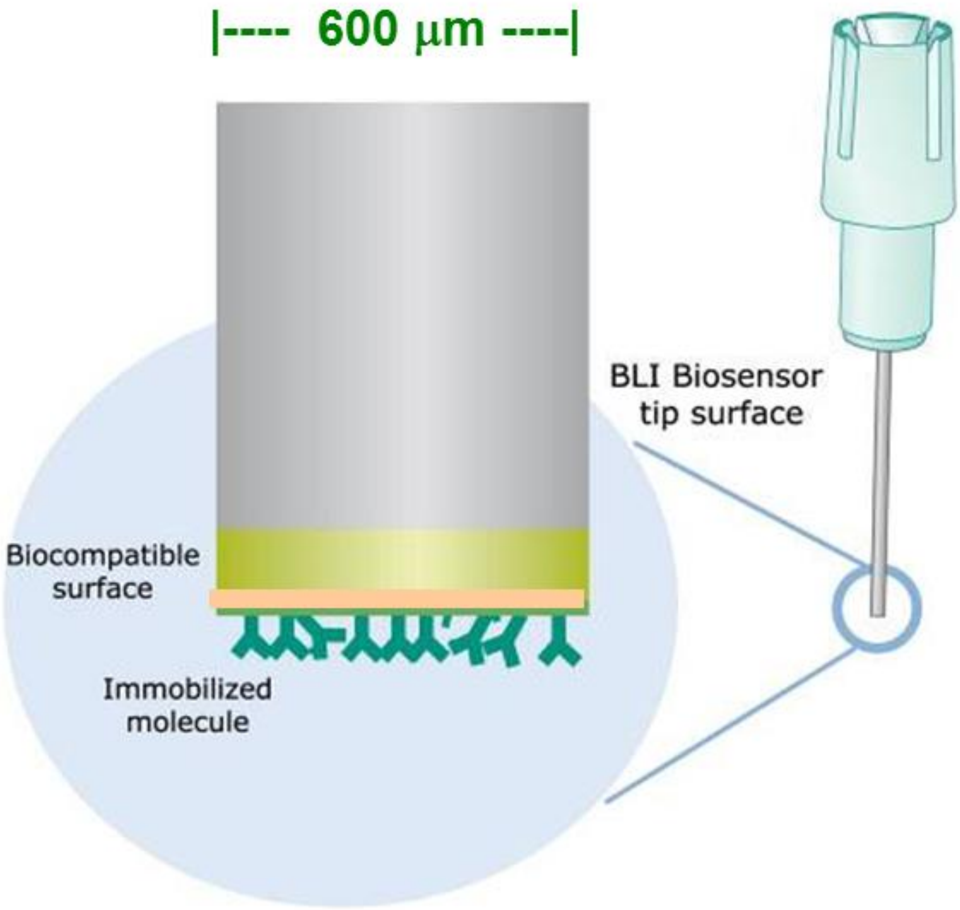
三大特点

- ✓ **无标记**
- ✓ **实时监测**
- ✓ **浸入即读**



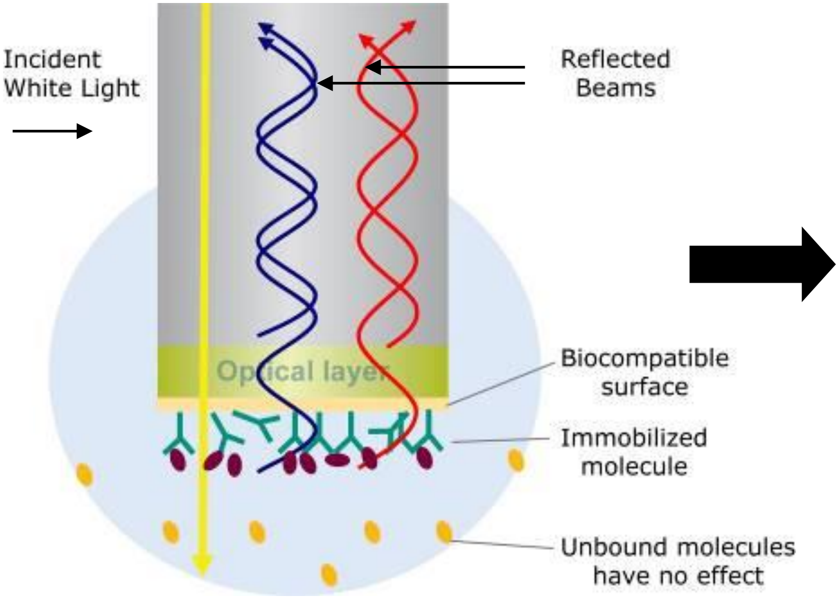


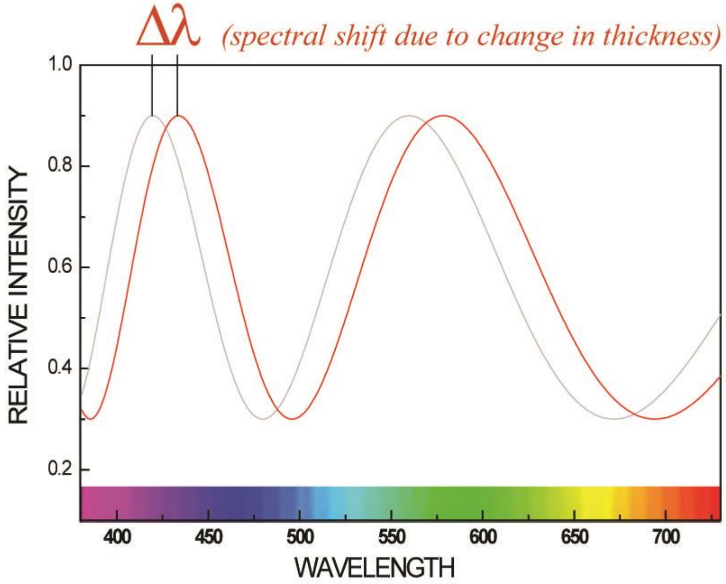
生物传感器（Biosensor）



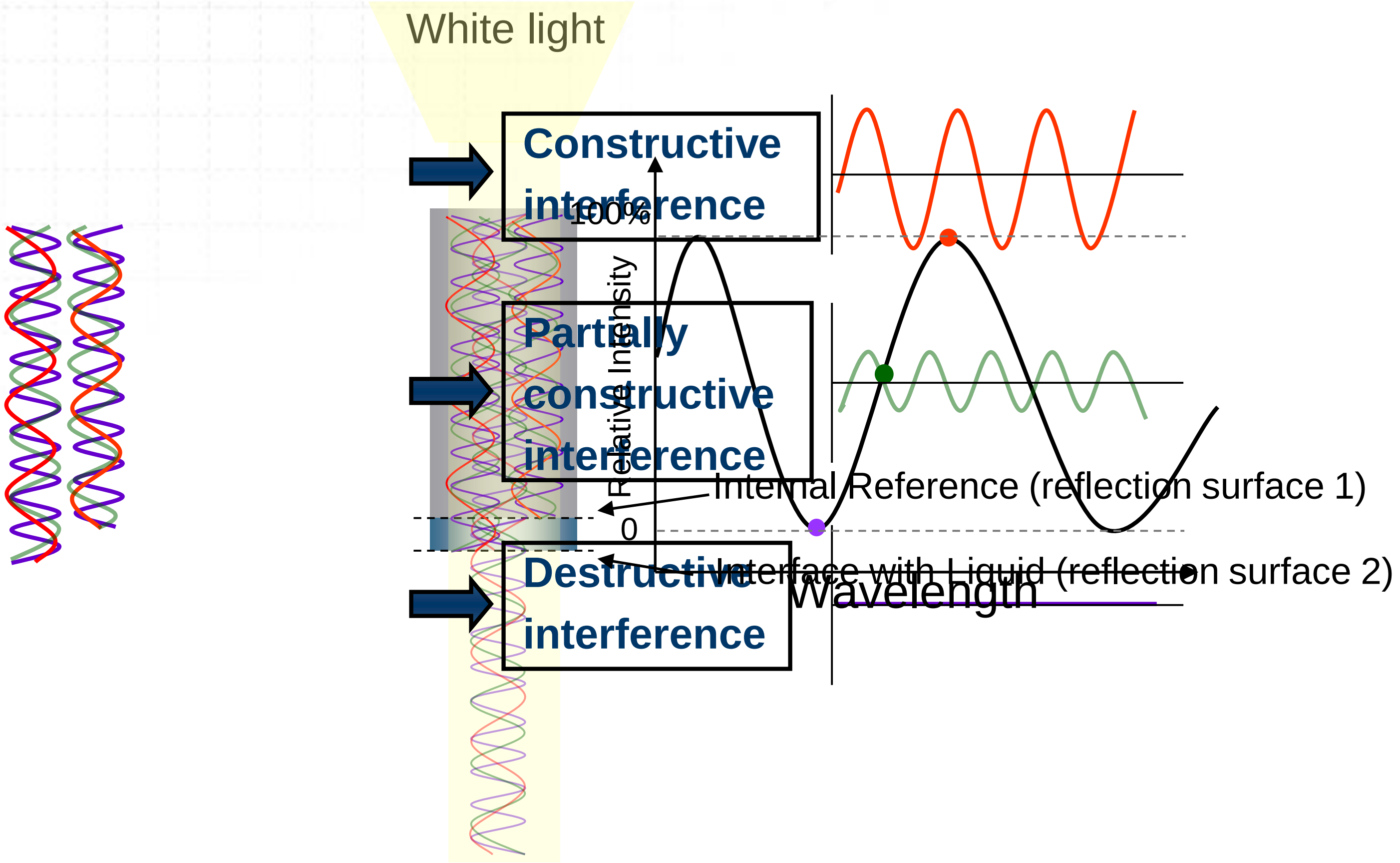
生物膜干涉（Bio-Layer Interferometry, BLI）

- 一束可见光穿过光纤，在传感器末端的光学膜层的两个界面会形成两束反射光谱，叠加形成一束干涉光谱
- 分子结合导致膜层厚度变化，并通过干涉光谱的位移值而体现

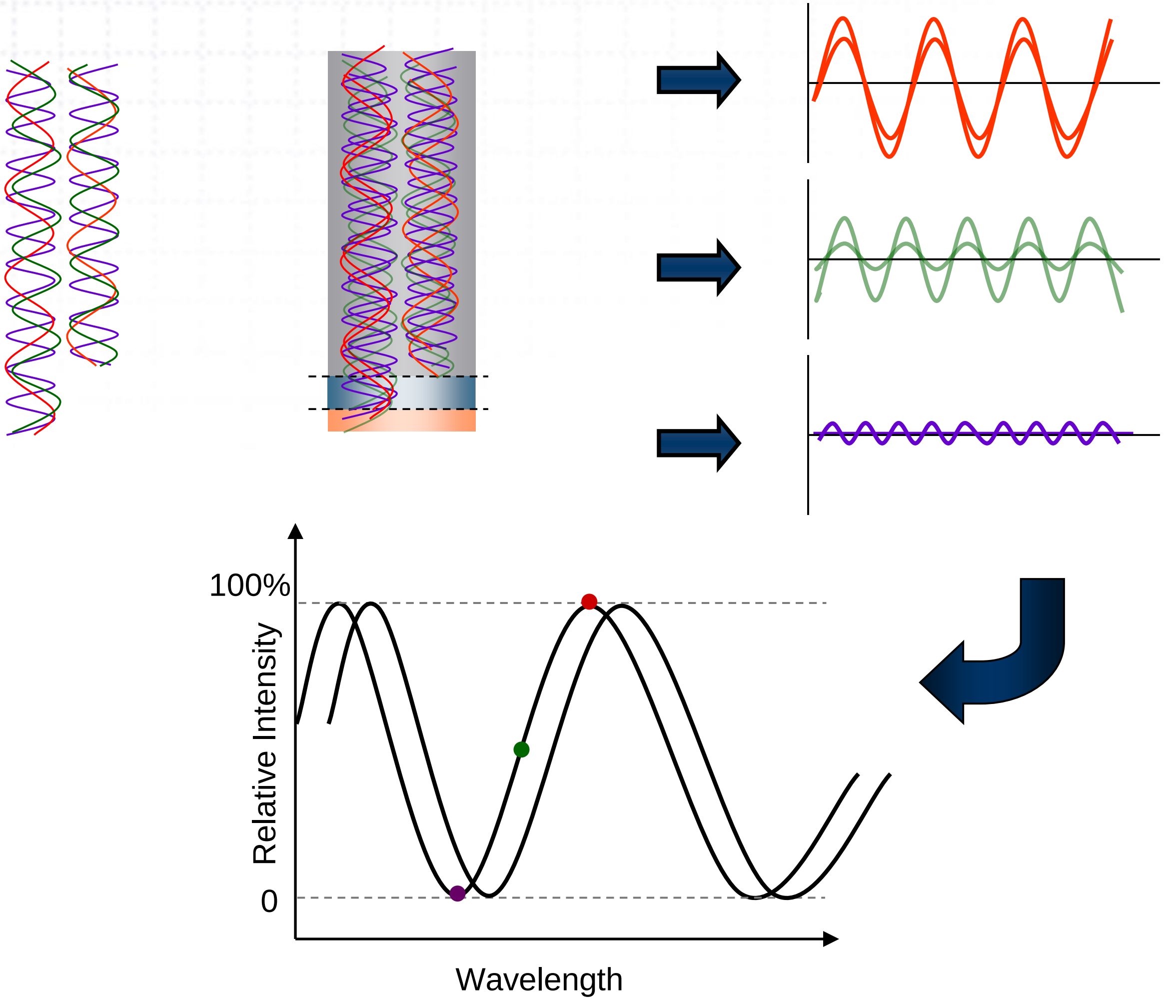




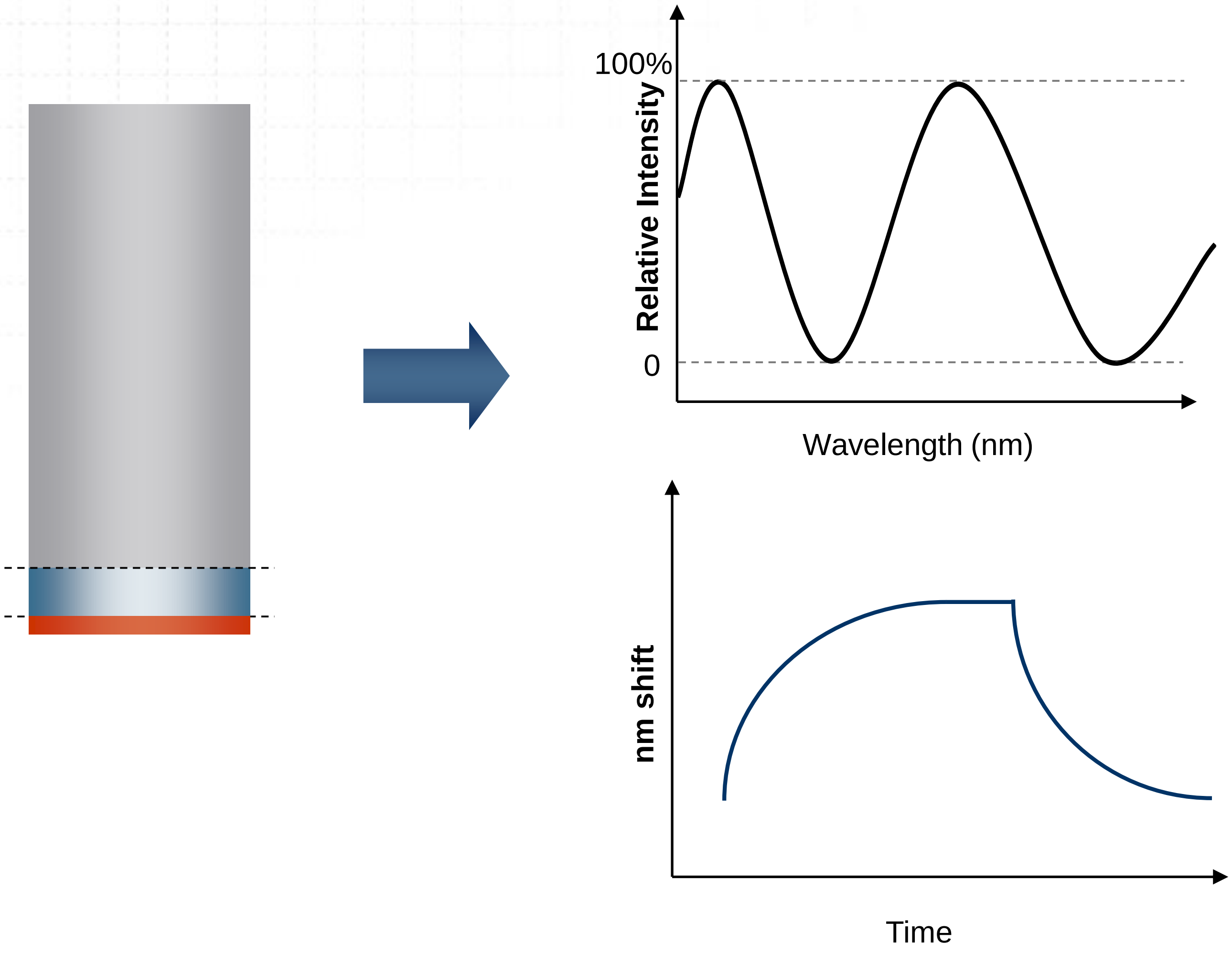
生物膜干涉（Bio-Layer Interferometry, BLI）



生物膜干涉（Bio-Layer Interferometry, BLI）



干涉光谱位移—时间曲线



丰富的传感器选择

通用型：

- Streptavidin (SA)
- Super Streptavidin (SSA)
- High Precision Streptavidin (SAX)
- Amine Reactive 2nd Generation (AR2G)
- Aminopropylsilane (APS)

捕获型:

- Anti-Penta-HIS (HIS1K)
- Anti-Penta-HIS 2G（His2）
- Ni-NTA (HIS)
- Anti-GST（GST）

抗体类：

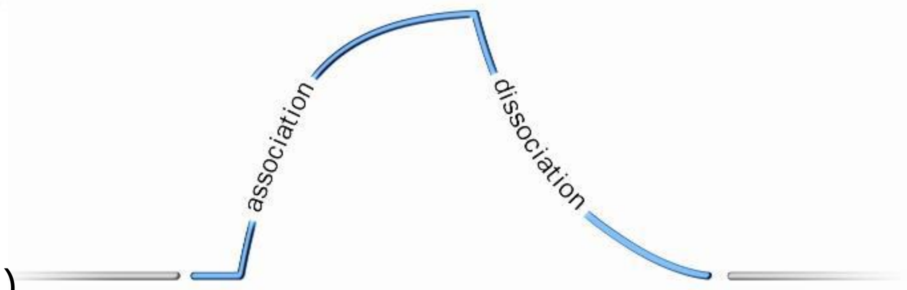
- Anti-Human IgG Fc (AHQ)
- Anti-Human IgG Fc (AHC)
- Anti-Murine IgG Fv (AMQ)
- Anti-Murine IgG Fc (AMC)
- Anti human IgG Fab（FAB2G）
- Protein A
- Protein G
- Protein L

✓ **再生20次以上**

Octet系统可以告诉你……

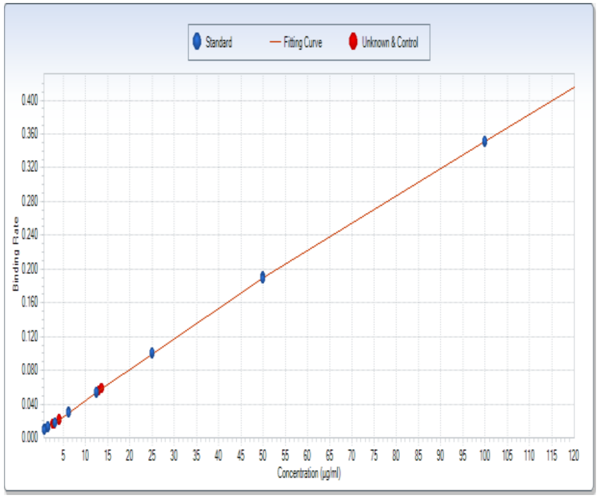
动力学测定

- 是否存在特异性结合（Yes / No）
- 结合亲和力（Affinity, K_D）
- 结合、解离速率（k_a , k_d）
- 筛选与排序（Screening / Ranking)
- 竞争实验（Competition Assay）
- 抗原表位配对（Epitope binning）



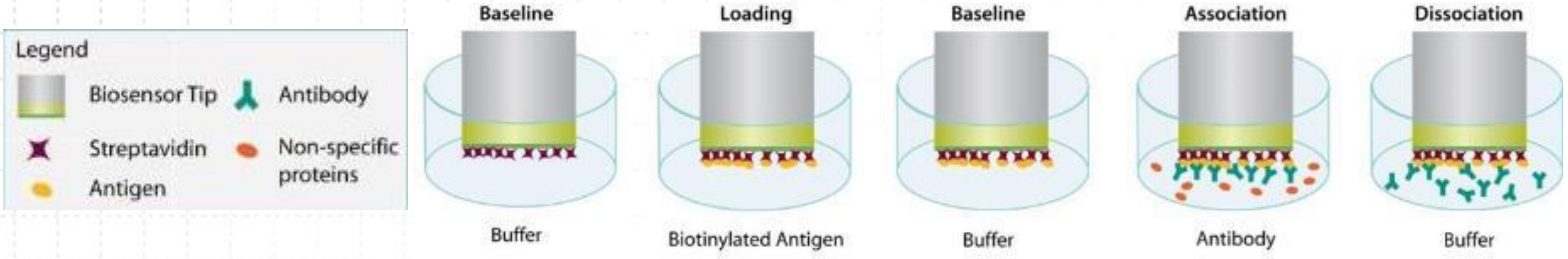
浓度测定

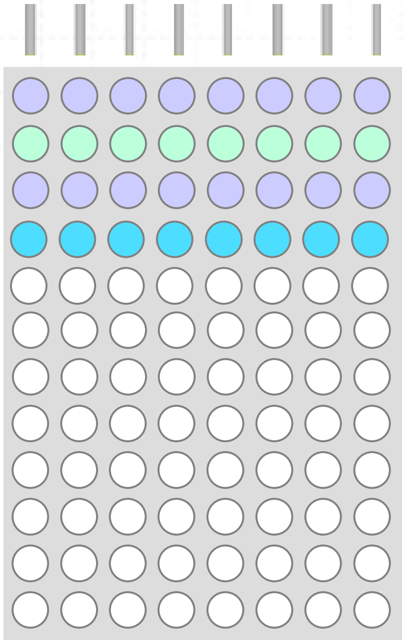
- 抗体或重组蛋白定量
- 抗生素或毒素检测
- ELISA定量替代实验
- 残留蛋白A、HCP检测等



PALL FortéBio®

快捷的动力学检测





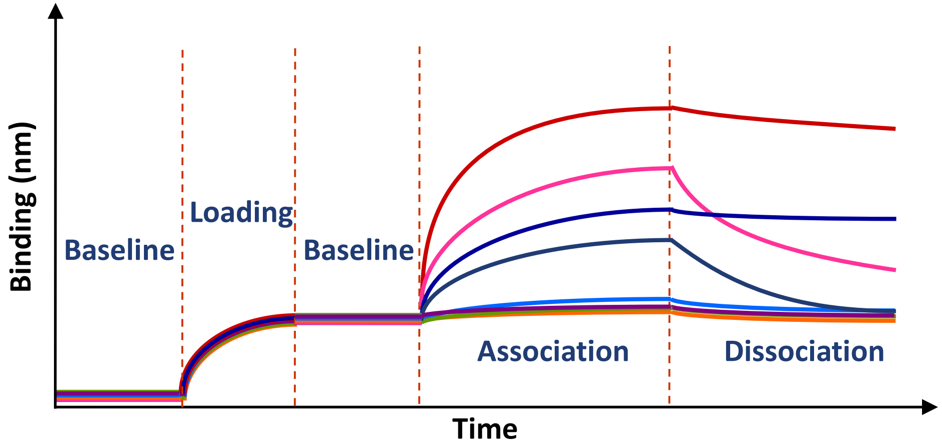
Biosensors

Buffer

ligand

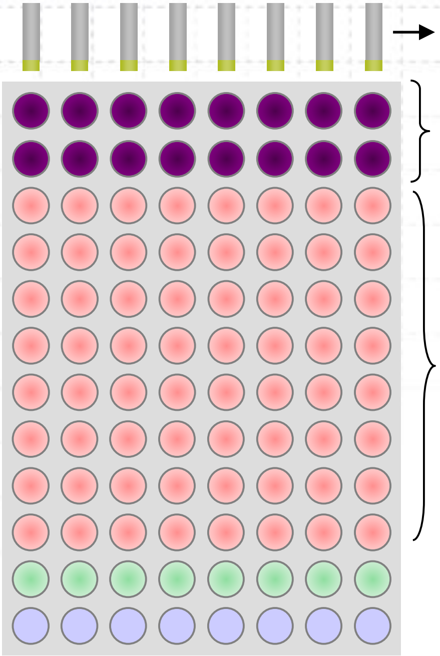
Buffer

Analyte



- 8个样品或者8个不同浓度同时平行检测
- 获取K_D，k_a，k_d等全面的动力学数据
- 显示每个步骤的实时曲线
- 可自定义任何步骤或方法

高通量的定量检测



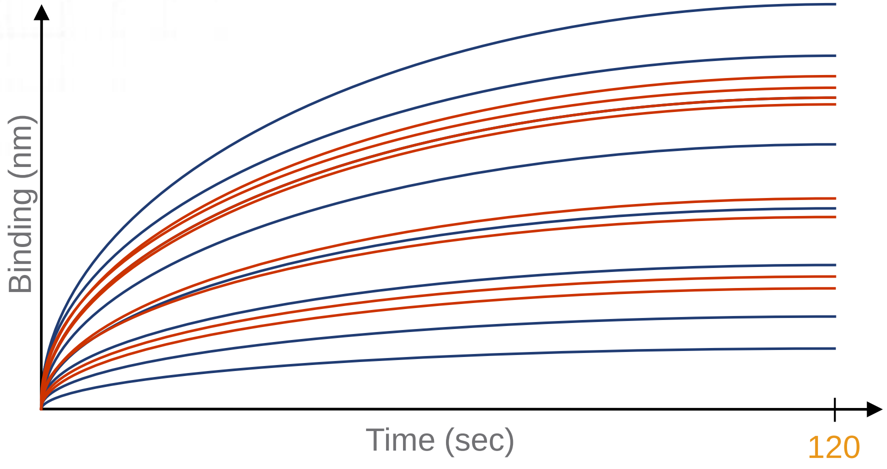
传感器

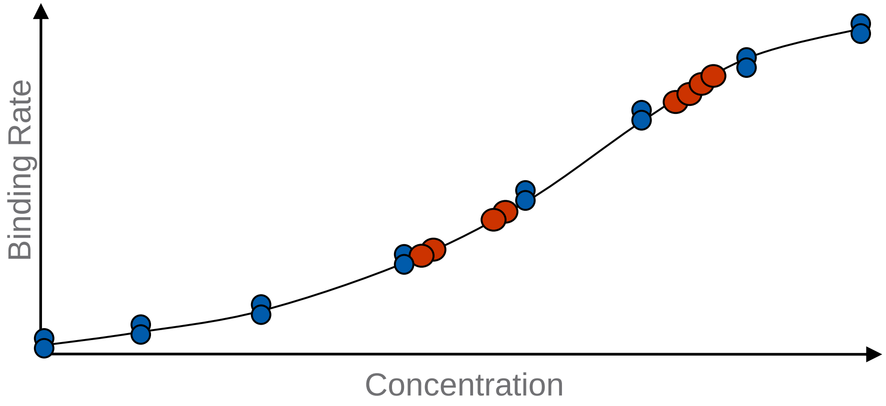
标准品

待测样品

再生液

中和液





- 动态检测范围：0.05 ~ 2000ug/mL
- 快速检测：96个样品15~30min
- 标准品可重复使用
- 检测重复性好：CV < 5%
- 适用于粗样品：细胞上清、培养液等

内 容

- 简介
- 原理
- **应用**
- 系统特点

应用领域广泛

COMPETITON/ INHIBITION ASSAYS

DNA/RNA BINIDNG PROTEINS

VIRUSES / VACCINES

LIGAND BINDING ASSAYS

ELISA REPLACEMENT

SMALL MOLECULES

BIOSIMILARS

APTAMERS

FC RECEPTORS

BINDING KINETICS

BIOPROCESSING

GLYCANS

应用领域广泛

动力学分析：

- 蛋白——蛋白
- 蛋白——小分子
- 脂类——蛋白/抗体
- 糖类——蛋白
- 肽类——蛋白
- 核酸——蛋白
- 病毒样颗粒——蛋白
- 纳米颗粒/材料
- 多分子相互作用
- 竞争实验
- 细胞实验

蛋白定量检测：

- 蛋白/抗体定量
- ELISA定量替代实验
- 病毒滴度分析
- 抗生素、毒素定量
- 残留蛋白A检测

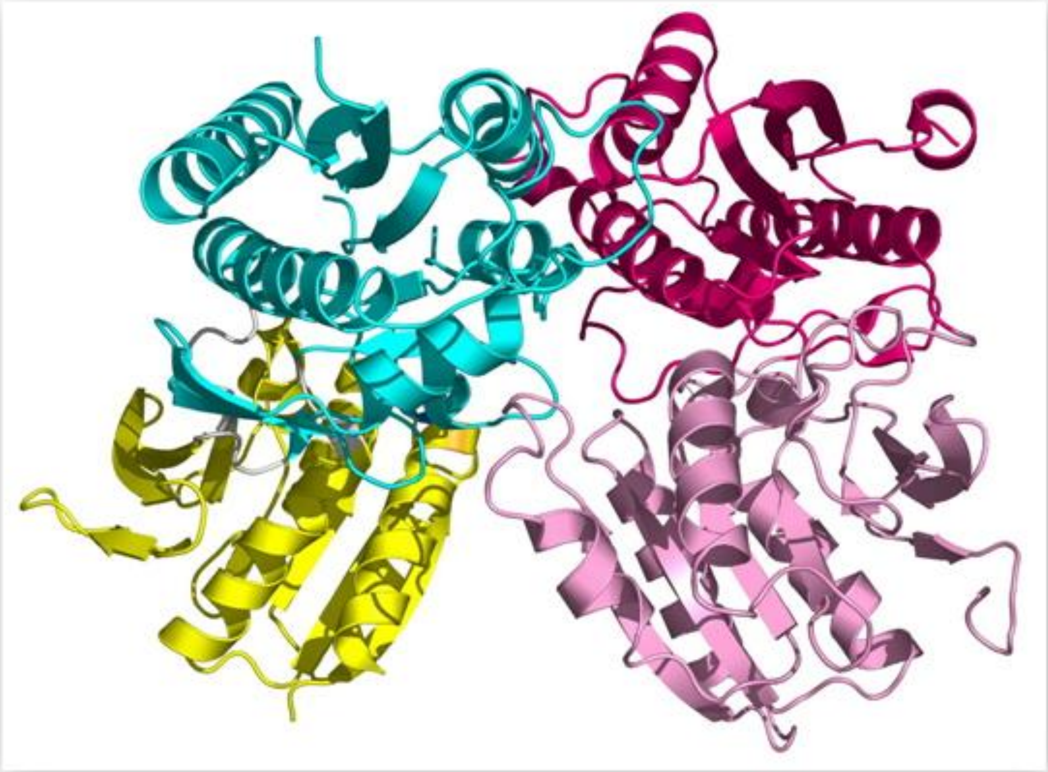
筛选实验：

- 晶体研究中蛋白筛选
- DNA适配子筛选
- 小分子片段筛选
- 次级筛选及Hit验证
- DNA-DNA错配检测
- 噬菌体展示技术
- 蛋白/多肽/小分子抑制试验
- 细胞克隆筛选
- 蛋白表达监测
- 生物反应器监测
- 抗原表位配对

实验开发：

- 培养基研发
- 生产工艺开发
- 抗体亚型分析
- 抗体配对

蛋白功能与结构



蛋白功能

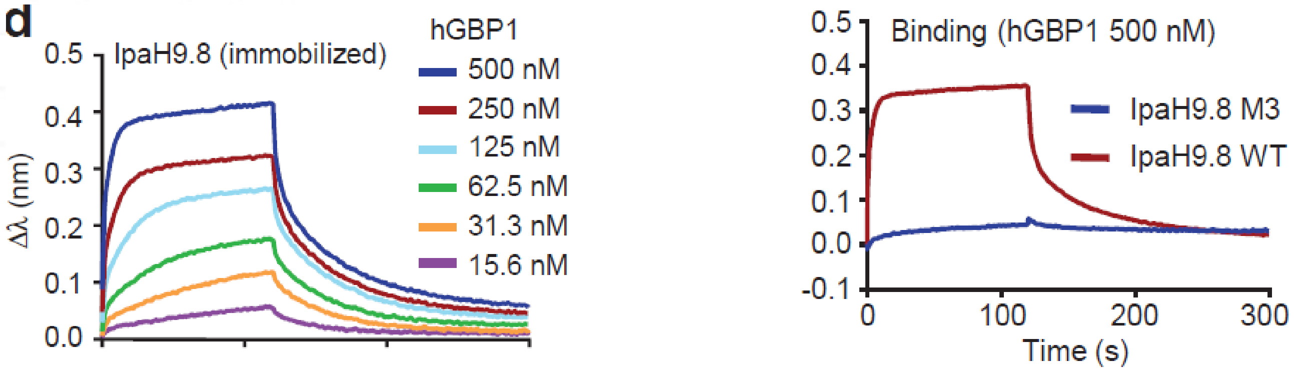
Ubiquitination and degradation of GBPs by a *Shigella* effector to suppress host defence

Peng Li, Wei Jiang, Qin Yu, Wang Liu, Ping Zhou, Jun Li, Junjie Xu, Bo Xu, Fengchao Wang and Feng Shao

nature

NIBS 邵峰 ，2017

研究揭示了志贺氏痢疾杆菌效应蛋白IpaH9.8通过泛素化降解宿主细胞内被干扰素调节的GBPs（鸟苷酸结合蛋白），从而抑制宿主的免疫反应并促进病原菌在宿主内的生存和增殖。



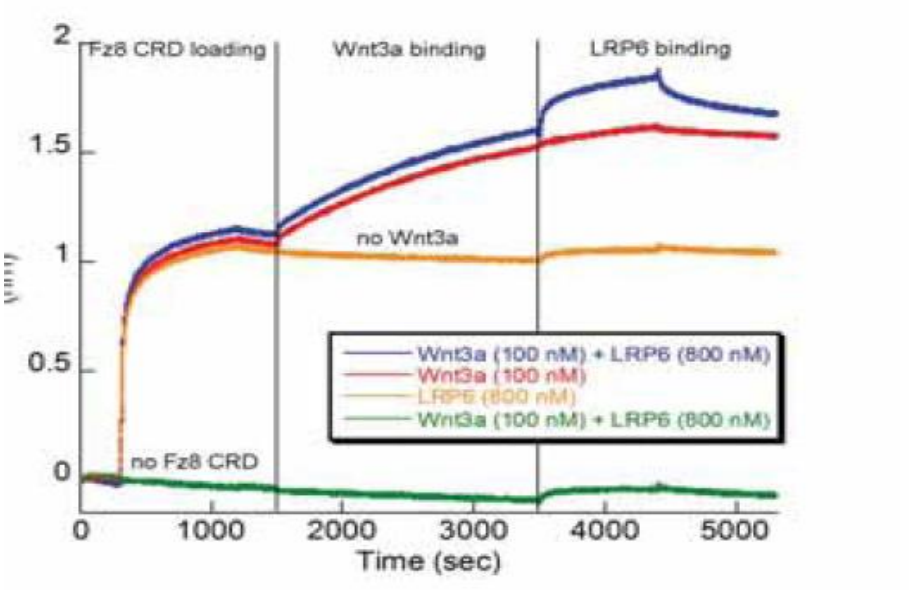
BLI及pulldown实验，结合细胞荧光成像检测发现，IpaH9.8可以直接结合在hGBP1蛋白上，导致hGBP1通过蛋白酶体通路被降解。当IpaH9.8被突变时，不能结合hGBP家族蛋白，hGBP1会定位到胞内志贺氏菌上来抑制其生长。动物实验表明，当被IpaH9.8基因缺失的志贺氏菌感染后，小鼠可以存活，而野生型志贺氏菌感染后的小鼠则死亡。

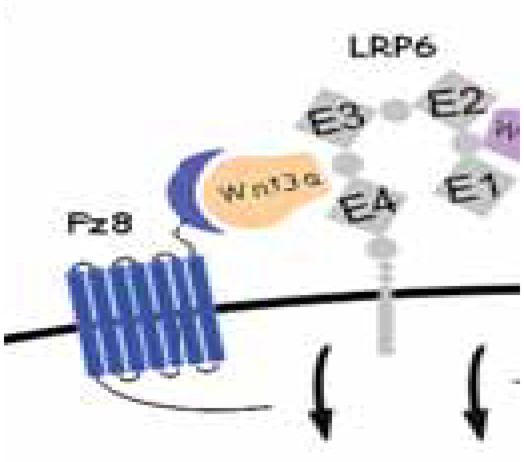
多个蛋白相互作用

Reconstitution of a Frizzled8-Wnt3a-LRP6 signaling complex reveals multiple Wnt and Dkk1 binding sites on LRP6

Eric Bourhis[1], Christine Tam[2], Yvonne Franke[2], J. Fernando Bazan[1],
James Ernst[3,1], Jiyoung Hwang[2], Mike Costa[4], Andrea G. Cochran[1] and Rami N. Hannoush[1]

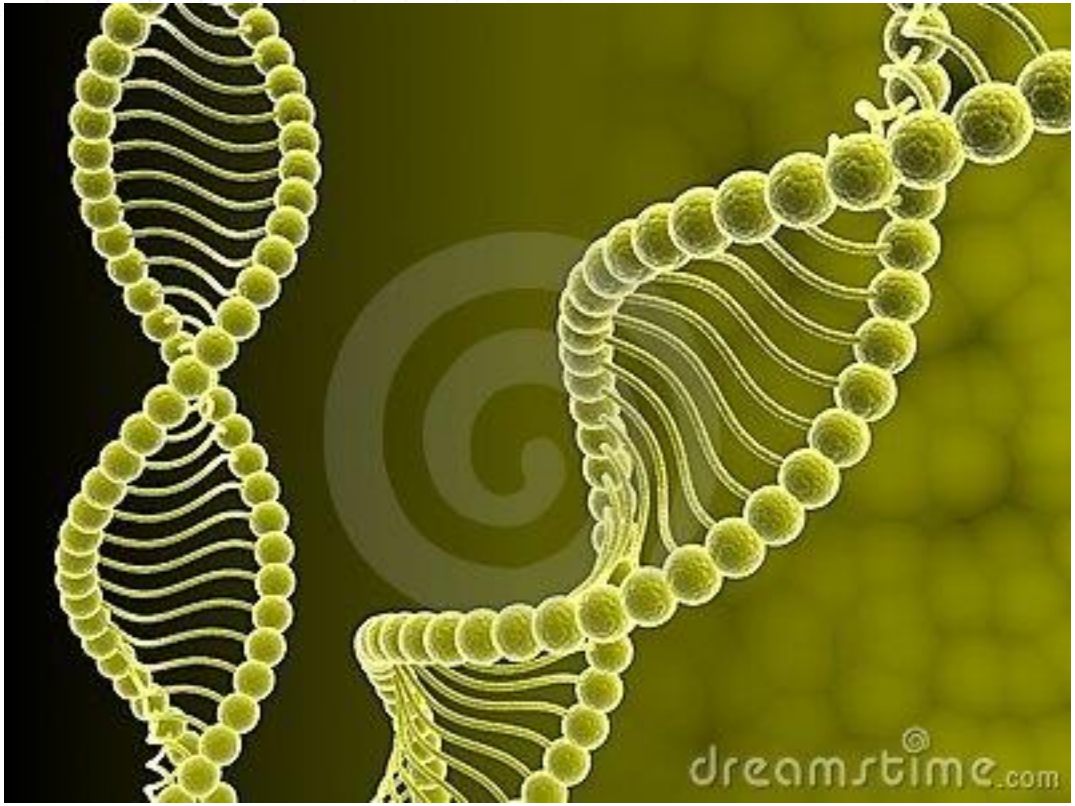
JBC，2010



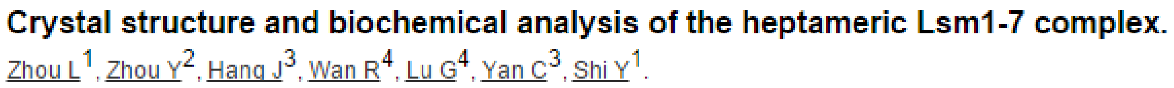


- Fz8与LRP6不能直接结合
- Wnt3a可以分别结合Fz8，LRP6
- Fz8-Wnt3a-LRP6复合物的形成中， Wnt3a起到关键作用

基因调控



RNA与蛋白

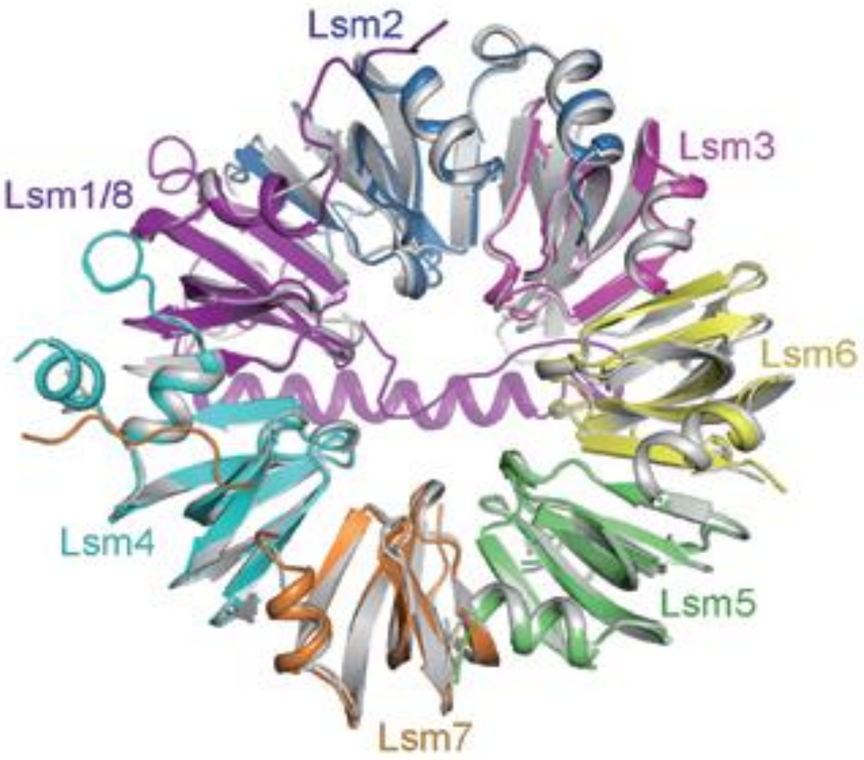


Crystal structure and biochemical analysis of the heptameric Lsm1-7 complex.

Zhou L[1], Zhou Y[2], Hang J[3], Wan R[4], Lu G[4], Yan C[3], Shi Y[1].

Cell Research

清华大学，2014



Lsm复合物在pre-mRNA的定向降解中起到非常重要的作用，有两种七聚体：Lsm1-7和Lsm2-8，分布在细胞核和细胞质中， 可以结合3'末端的U6 snRNA（小核RNA ），从而在RNA的代谢中起着重要作用。

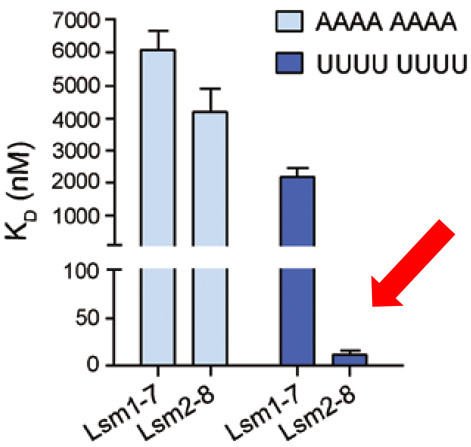
BLI技术研究Lsm复合物与mRNA末端寡聚核糖核酸（AAAAAAAA和UUUUUUUU）的结合能力和机制。

方法与结果

链霉亲和素传感器（SA）

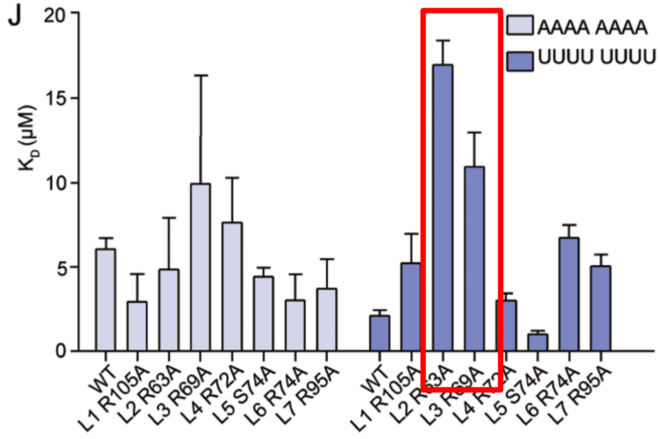
生物素化RNA

Lsm蛋白



Lsm1-7与8A和8U的亲和力分别为6uM和2uM，没有明显差别。

Lsm2-8与8U的结合明显强于8A（200倍，4uM与20nM），说明Lsm2-8与8U是选择性结合的。



Lsm1-7 突变体与RNA的相互作用

L2，L3上的Arg突变后，Lsm1-7复合物与8U的结合能力显著下降

各种突变与与8A的结合能力无显著变化。

DNA与蛋白

Genome-wide studies identify a novel interplay between AML1 and AML1/ETO in t(8;21) acute myeloid leukemia

Yizhen Li,[1,*] Huanwei Wang,[1,*] Xiaoling Wang,[1] Wen Jin,[1] Yun Tan,[1] Hai Fang,[2] Saijuan Chen,[1,2] Zhu Chen,[1-3] and Kankan Wang[1-3]

blood

上海血研所，2016

Probes containing the short AML1 motifs

S1: 5'-TCCGGTGGTGC ***TGTGGT*** CTGCCCCTGGAGA-3'
3'-AGGCCACCACG ***ACACCA*** GACGGGGACCTCT-5'

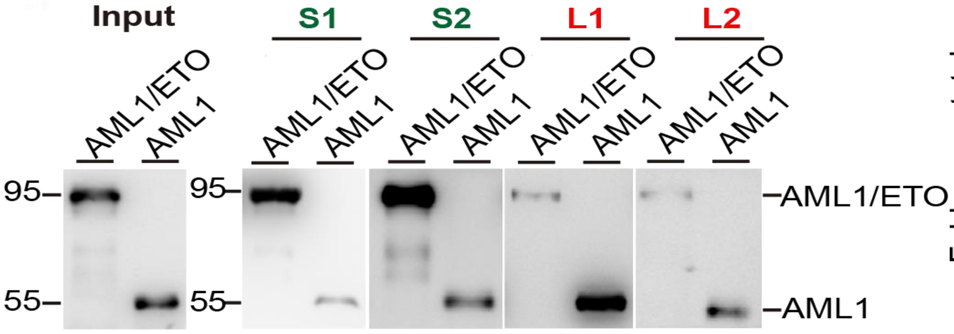
S2: 5'-ACCACCTGCATT ***ACCACA*** GCCTCCACGGTG-3'
3'-TGGTGGACGTAA ***TGGTGT*** CGGAGGTGCCAC-5'

Probes containing the long AML1 motifs

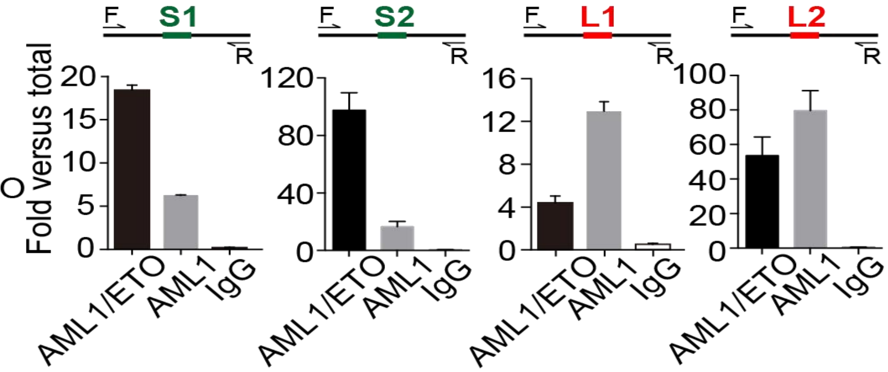
L1: 5'-GTGAGTACCCA***TGTGGTTT***AACGCTTGACT-3'
3'-CACTCATGGGT***ACACCAAA***TTGCGAACTGA-5'

L2: 5'-GCAGAAGGAAGC ***TGTGGTTT*** CTGGTCTTCCT-3'
3'-CGTCTTCCTTCG ***ACACCAAA*** GACCAGAAGGA-5'

In vitro DNA pull-down

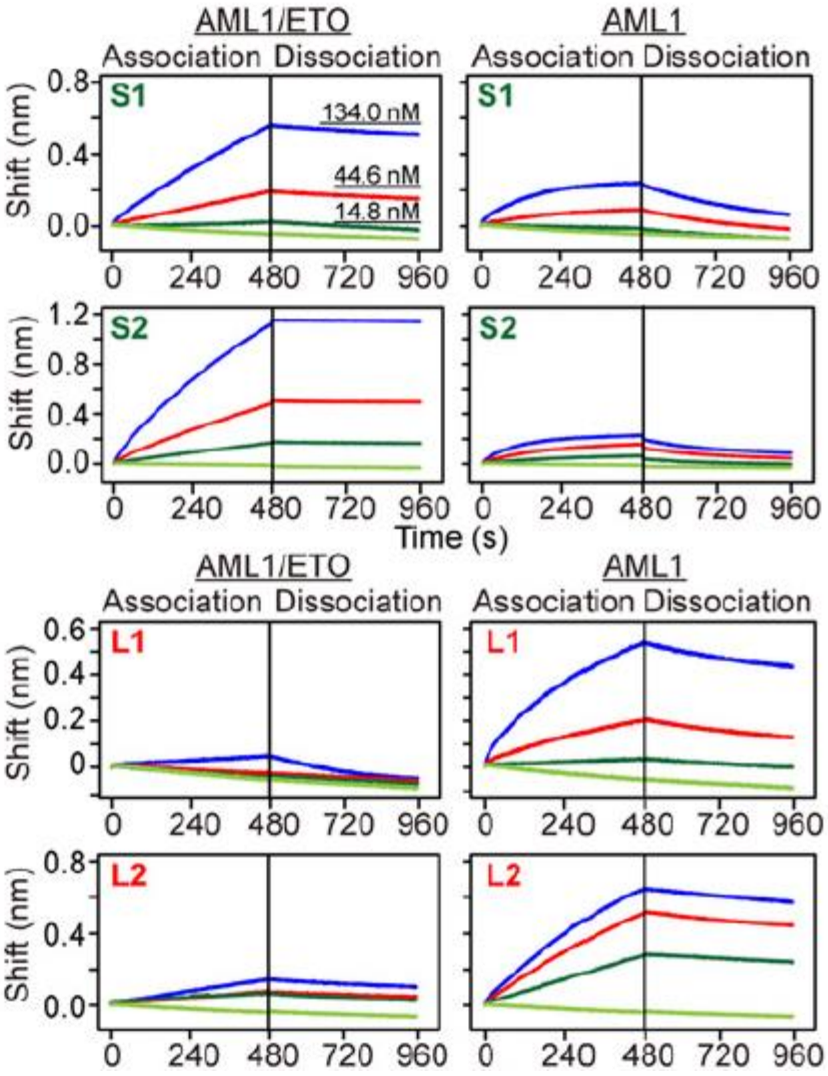


In vivo ChIP-qPCR



Blood审稿人要求提供亲和力数据，但是pull-down和ChIP可以定性结果，无法计算序列与AML1/ETO及AML1的具体亲和力

DNA与蛋白

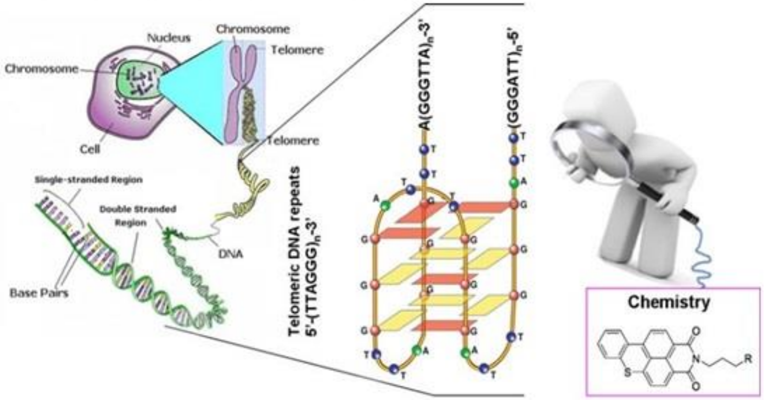


	KD (M)	
	AML1/ETO	AML1
S1	2.37E-09	4.55E-06
S2	3.33E-10	6.70E-07
L1	1.32E-04	1.08E-06
L2	1.38E-07	2.57E-09

SA传感器固化biotin-AML1基序S1，S2，L1和L2，分别与不同浓度AML1/ETO和AML1进行结合与解离得到KD。

发现AML1/ETO与S结合强于AML1，而AML1与L结合强于AML1/ETO，这个结合与传统方法的结果一致，但是能定量。

小分子及天然产物研究


Nucleus
Chromosome
Chromosome
Telomere
Cell
Telomere
Single-stranded Region
Double Stranded Region
DNA
Base Pairs
Telomeric DNA repeats
5'-(TTAGGG)n-3'
A(GGGTTA)n-3'
(GGGATT)n-5'
Chemistry
S
N
O
O
R

小分子激动剂

Small-molecule modulation of Wnt signaling via modulating the Axin-LRP5/6 interaction

Sheng Wang[1,5], Junlin Yin[2,5], Duozhi Chen[2], Fen Nie[1], Xiaomin Song[1], Cong Fei[1], Haofei Miao[1], Changbin Jing[3], Wenjing Ma[3], Lei Wang[2], Sichun Xie[1], Chen Li[4], Rong Zeng[4], Weijun Pan[3], Xiaojiang Hao[2]* & Lin Li[1]*

nature
chemical biology

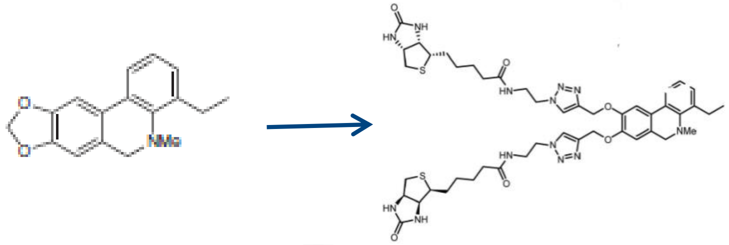
中科院上海生化所，2013年

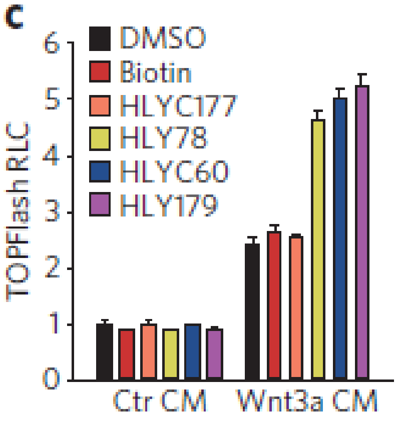
Wnt信号通路的异常激活与癌症的发生及发展紧密联系。

石蒜碱衍生物---小分子HLY78，可以增强靶蛋白Axin与Wnt信号共受体LRP6的结合，促进了LRP6的磷酸化，提高Wnt信号通路的激活效率, HLY78 可作为一种潜在的药物先导化合物。

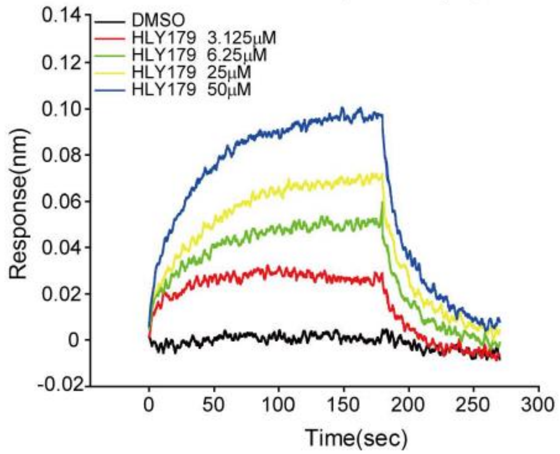
研究发现HLY78的衍生物HLY179在细胞学实验中表现出更好的活性。经Octet检测，HLY179和DAX的亲和力为8.83uM，高于HLY78。

HLY78 HLY179

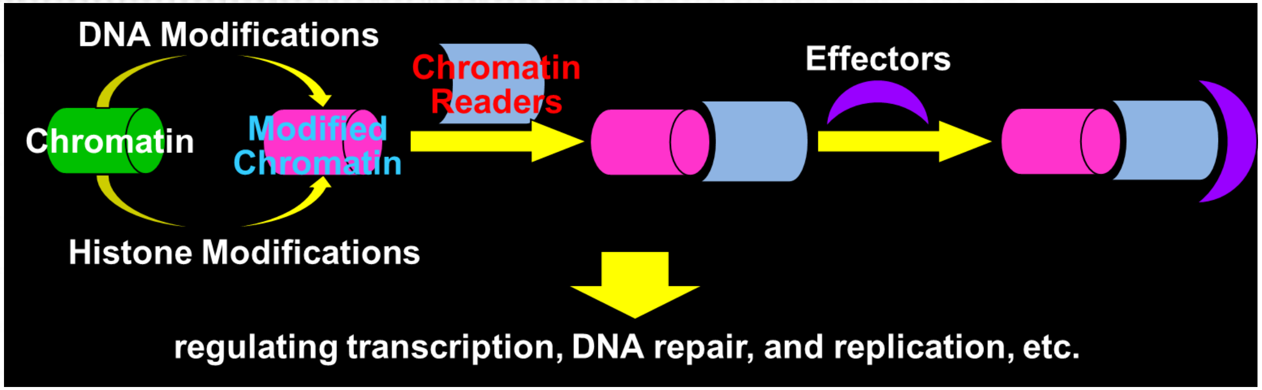




DAX V.s. HLY179(KD=8.83 μM)



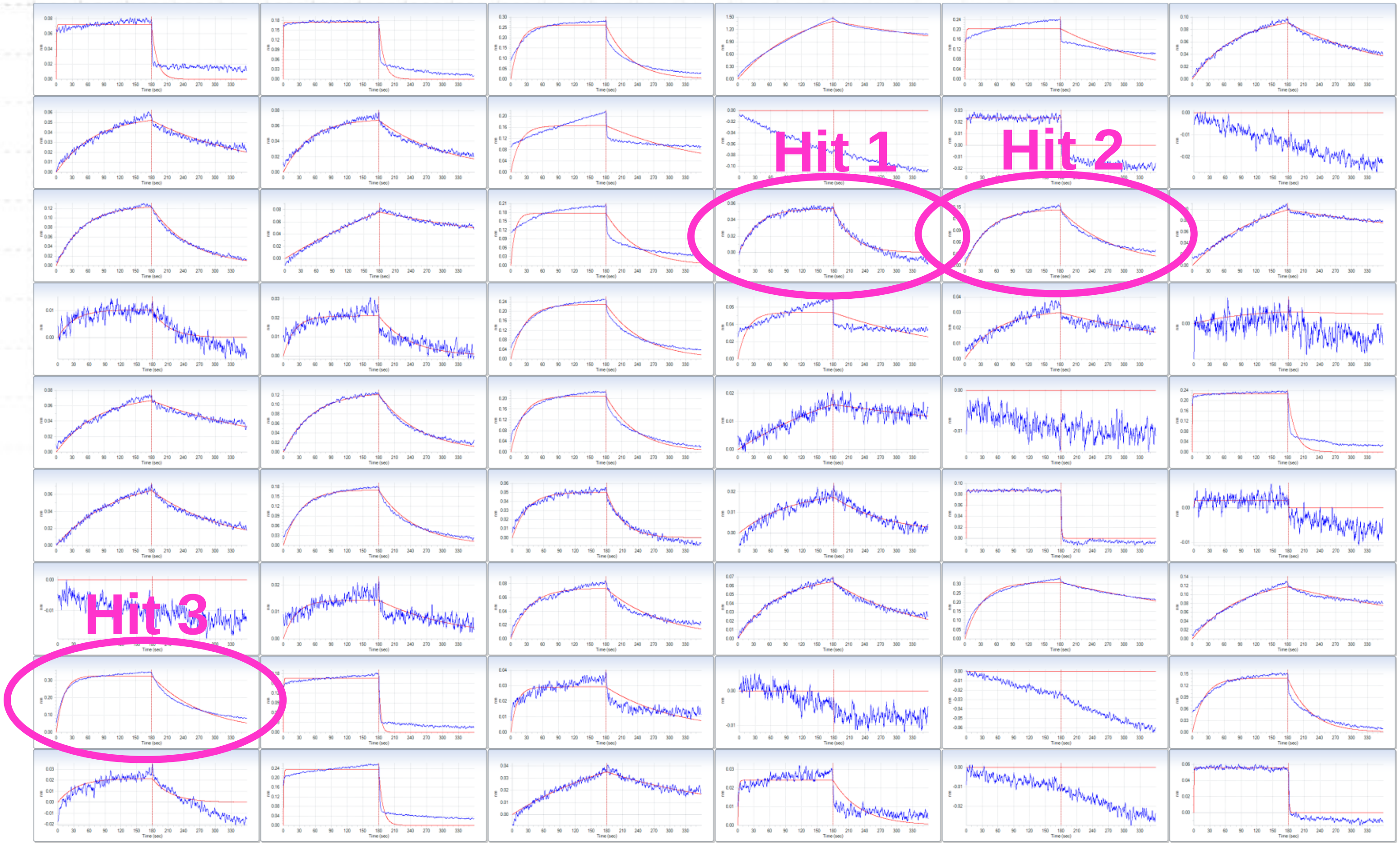
小分子化合物筛选及鉴定实例



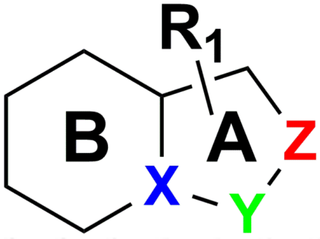
溴结构域Bromodomain (BRD) 蛋白

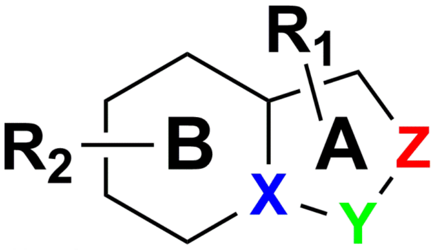
- 染色质修饰 “Reader” 蛋白家族
- 特异性结合乙酰化组蛋白并招募下游 “Effectors” (染色质修饰/重塑酶等)
- BRD蛋白异常调控已经被证实在多种疾病中起着重要作用：心血管，肿瘤，感染，炎症，胆固醇调控…

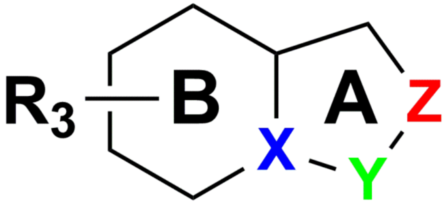
Hits单浓度筛选



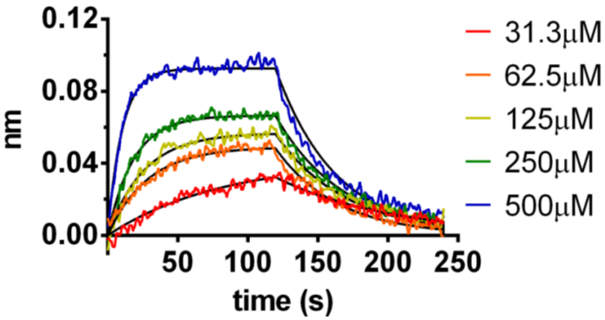
多浓度梯度验证



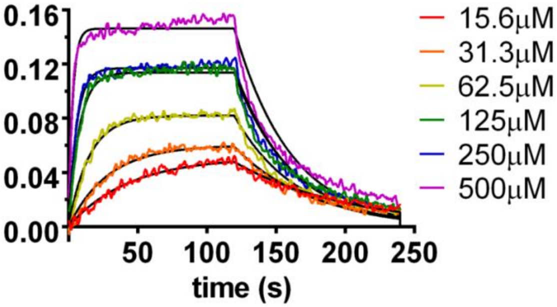




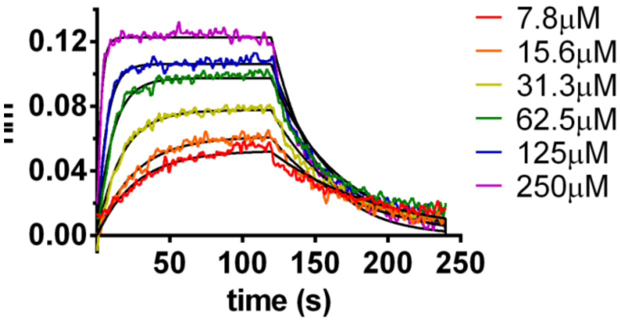
Hit 1/BRD4BD1



Hit 2/BRD4BD1



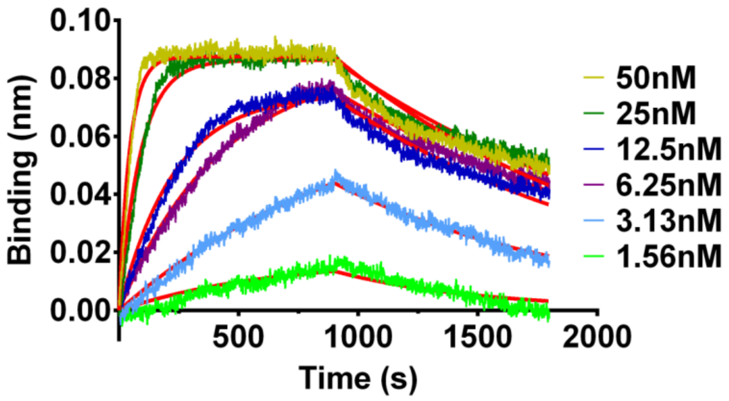
Hit 3/BRD4BD1

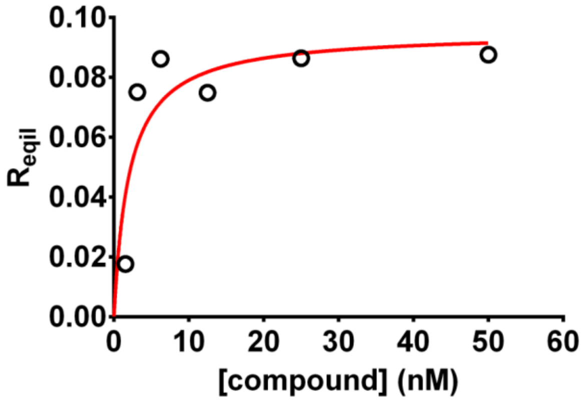


CMPD	MW（Da）	K_D (μM)	k_a (1/Ms)	k_d (1/s)
Hit 1	133	89.2	2.21E+02	1.97E-02
Hit 2	210	42.5	5.57E+02	2.37E-02
Hit 3	211	14.9	1.52E+03	2.27E-02

Leads优化

	BRD2 BD2	BRD3 BD2	BRD4 BD2
CMPD 5	12.7±4.2		13.9±2.0
CMPD 6	4.1±0.7	4.5±0.9	14.4±1.1
CMPD 7	3.5±0.7	1.5±0.3	3.6±0.1





- 优化得到高亲和力nM级别的候选化合物

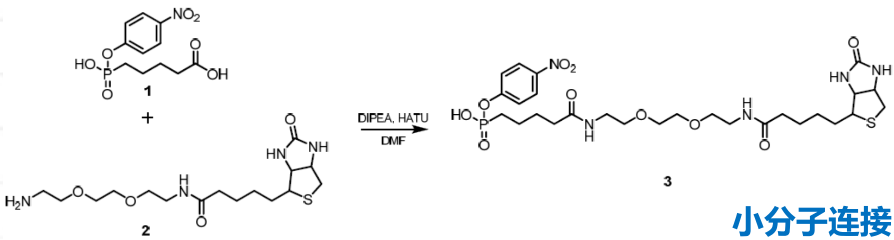
固化小分子检测蛋白

Mutational Analysis of 48G7 Reveals that Somatic Hypermutation Affects Both Antibody Stability and Binding Affinity

Sophie B. Sun,[†] Shiladitya Sen,[§] Nam-Jung Kim,[†] Thomas J. Magliery,[*,§] Peter G. Schultz,[*,†] and Feng Wang[*,‡]

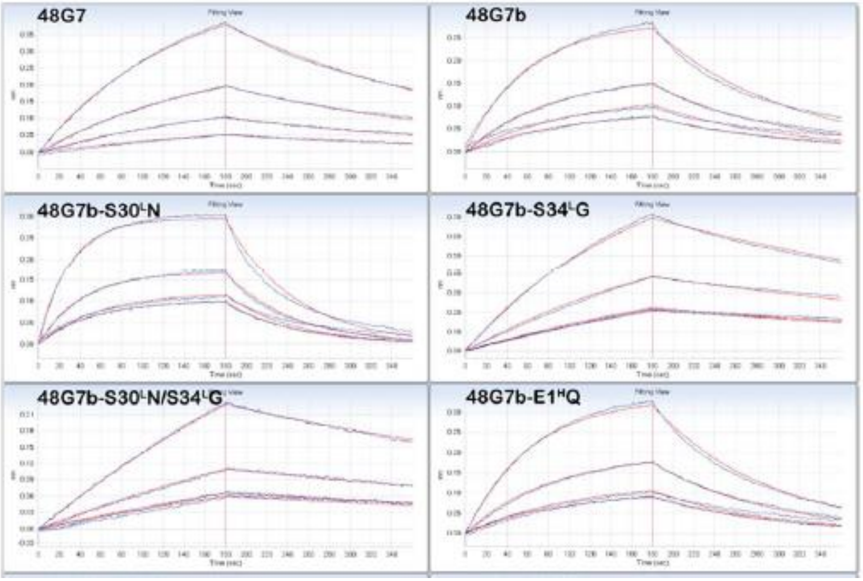
JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

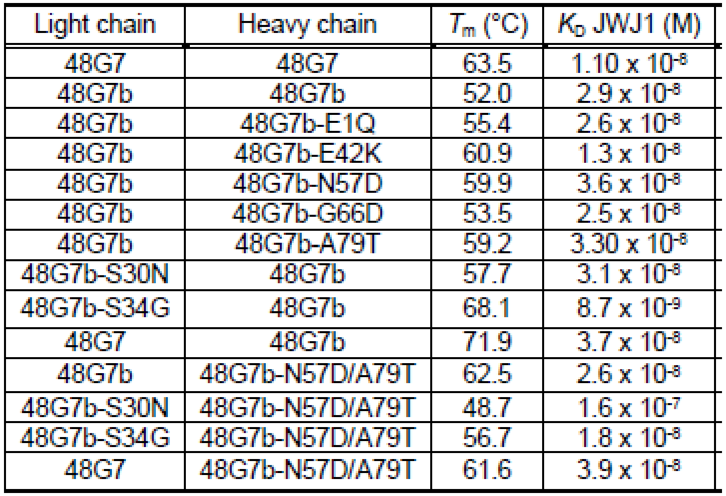
The Scripps研究所，2013



小分子连接biotin

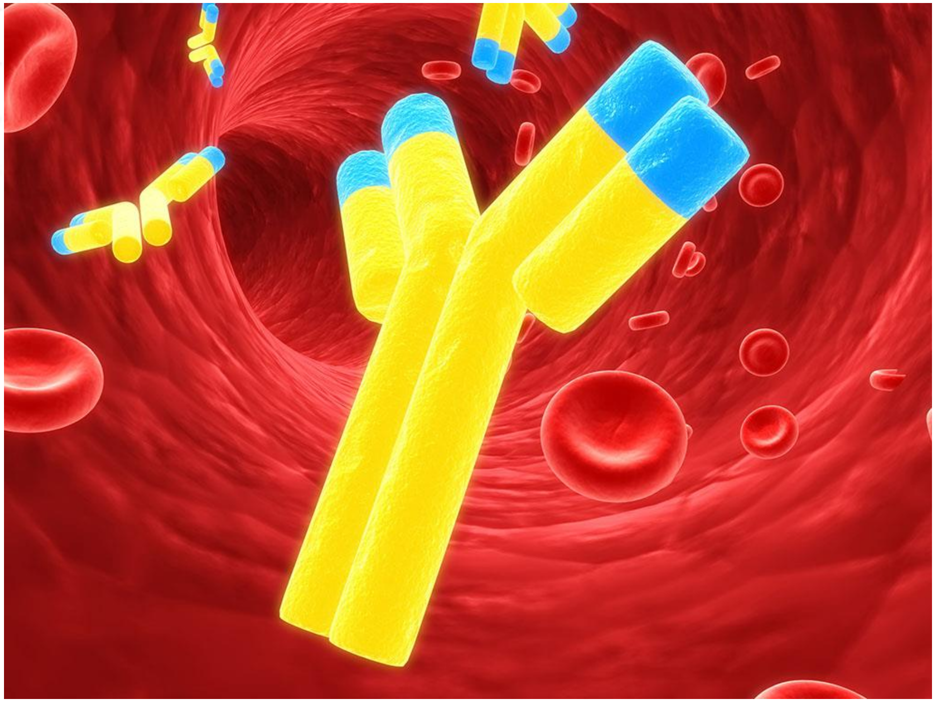
Scheme S1. Conjugation of JWJ1 phosphonate hapten to Amine-PEG2-Biotin



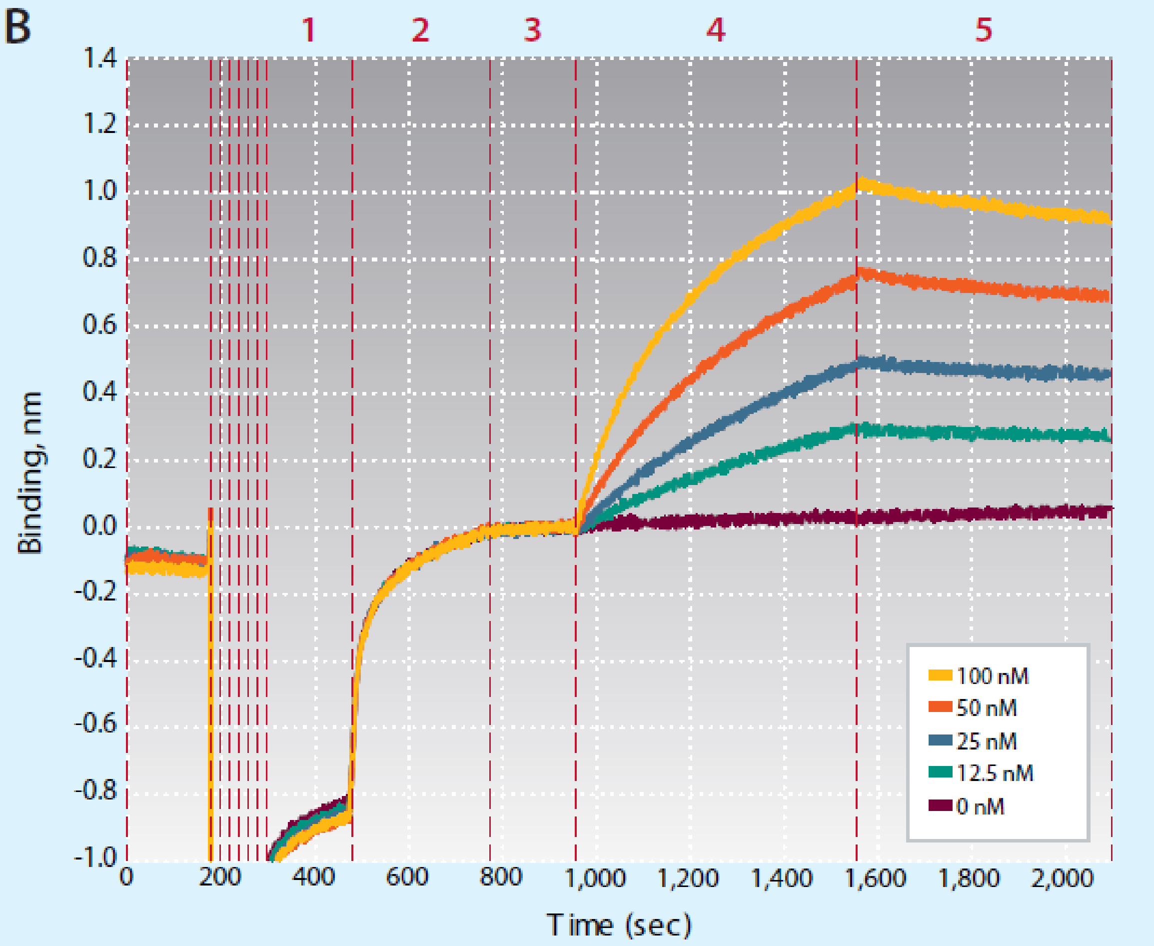


Light chain	Heavy chain	T_m (°C)	K_D JWJ1 (M)
48G7	48G7	63.5	1.10 x 10^{-8}
48G7b	48G7b	52.0	2.9 x 10^{-8}
48G7b	48G7b-E1Q	55.4	2.6 x 10^{-8}
48G7b	48G7b-E42K	60.9	1.3 x 10^{-8}
48G7b	48G7b-N57D	59.9	3.6 x 10^{-8}
48G7b	48G7b-G66D	53.5	2.5 x 10^{-8}
48G7b	48G7b-A79T	59.2	3.30 x 10^{-8}
48G7b-S30N	48G7b	57.7	3.1 x 10^{-8}
48G7b-S34G	48G7b	68.1	8.7 x 10^{-9}
48G7	48G7b	71.9	3.7 x 10^{-8}
48G7b	48G7b-N57D/A79T	62.5	2.6 x 10^{-8}
48G7b-S30N	48G7b-N57D/A79T	48.7	1.6 x 10^{-7}
48G7b-S34G	48G7b-N57D/A79T	56.7	1.8 x 10^{-8}
48G7	48G7b-N57D/A79T	61.6	3.9 x 10^{-8}

免疫学研究



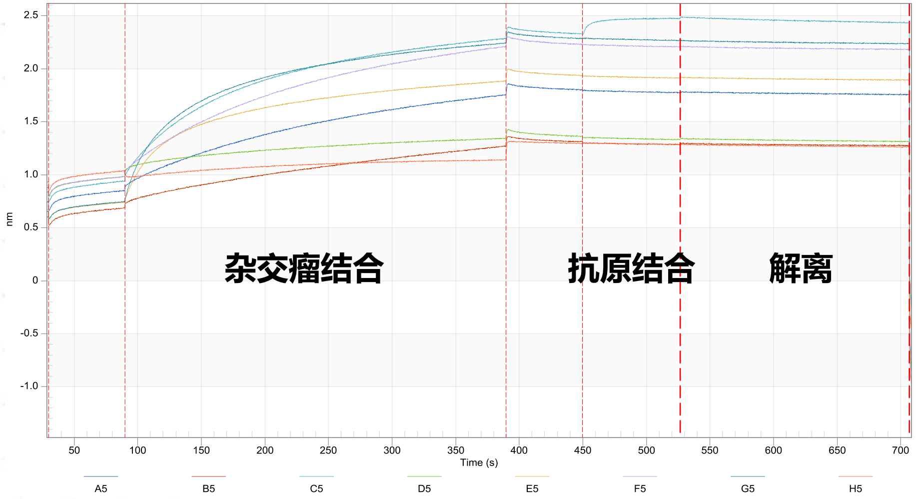
抗体动力学分析

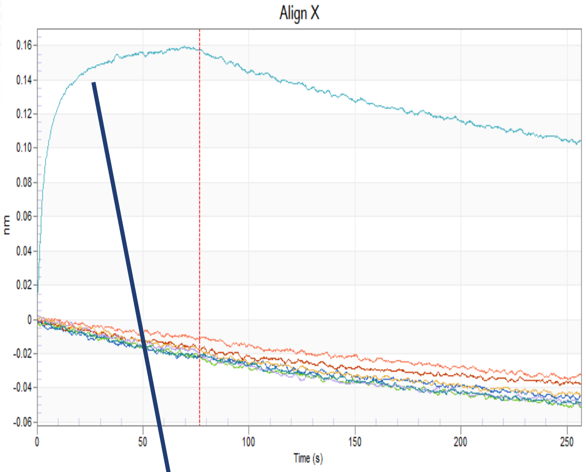


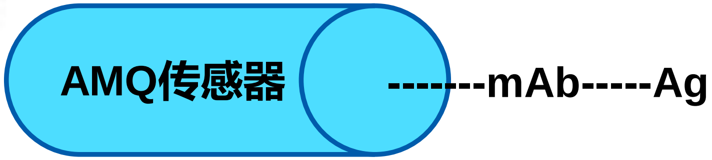
D

K_D (M)	k_{on} (1/Ms)	k_{on} Error	k_{dis} (1/s)	k_{dis} Error
5.34E-09	4.70E+04	1.60E+02	2.51E-04	1.78E-06

杂交瘤筛选







KD (M)	kon(1/Ms)	kdis(1/s)
2.12E-09	4.53E+05	9.61E-04

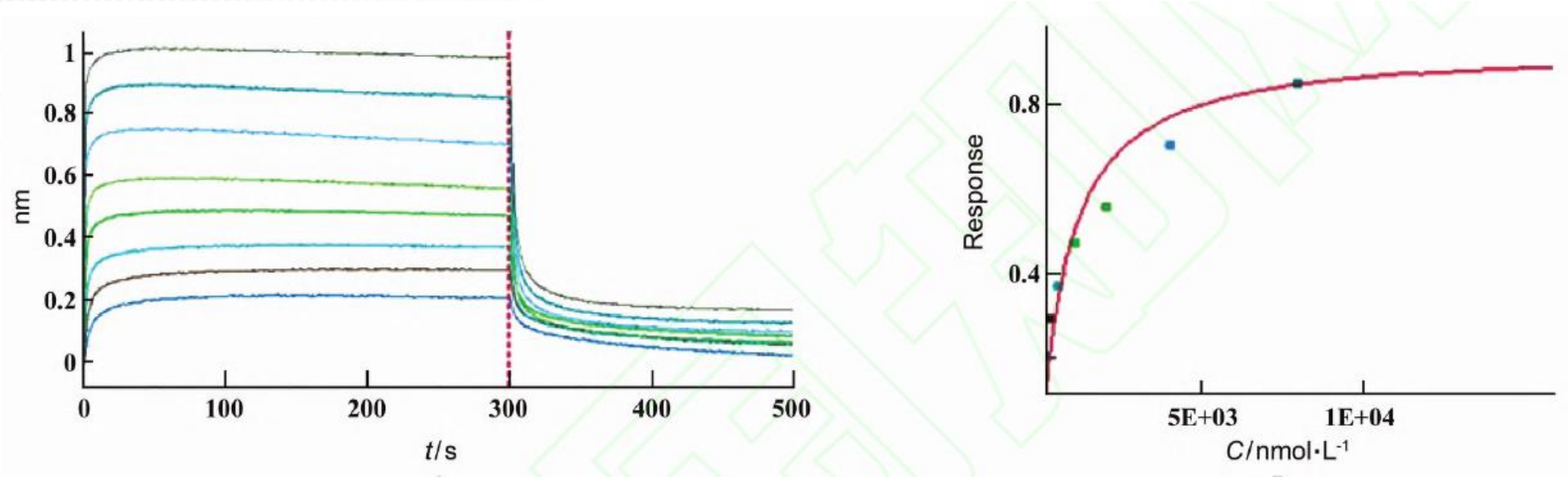
- 将杂交瘤上清（单克隆抗体）固定在AMQ传感器表面，与抗原进行结合解离
- 8个样品测试只需12min即可完成，AMQ传感器可再生使用10次以上
- 同时，可以通过杂交瘤上清（抗体）的固化速率判断目标抗体的浓度

抗体与受体

· 生物医药前沿 ·

应用生物膜干涉技术测定治疗性单抗与新生儿 Fc 受体的亲和力

张 峰，武 刚，于传飞，王文波，高 凯，王 兰
（中国食品药品检定研究院单克隆抗体产品室 卫生部生物技术产品检定及标准化重点实验室，北京 100050）



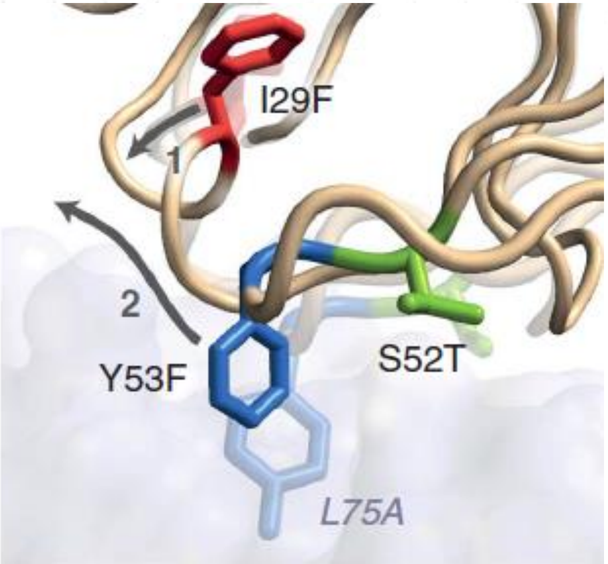
- BLI技术已经成为检测FcRn与抗体的亲和力的标准方法

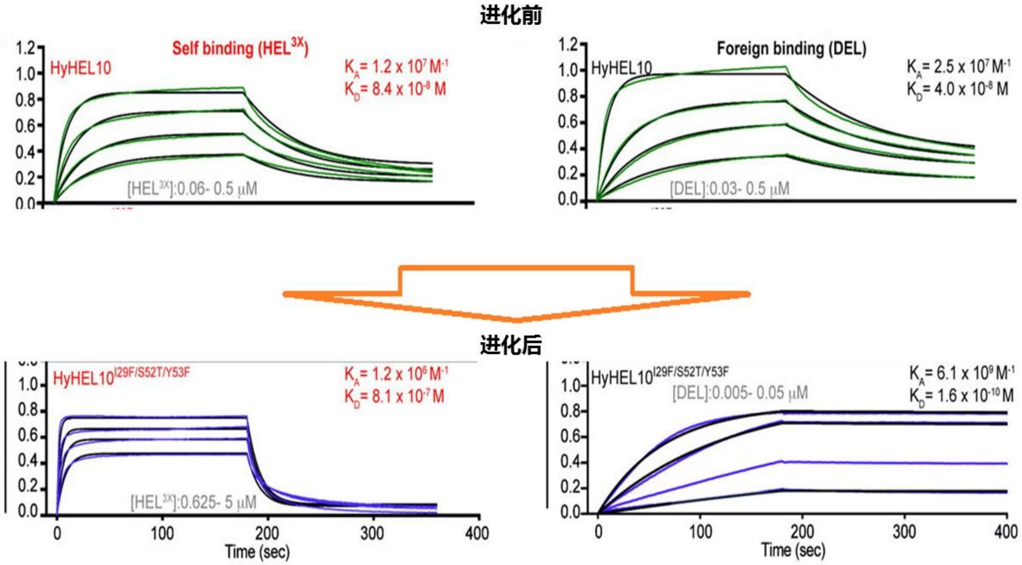
抗体亲和力

Germinal center antibody mutation trajectories are determined by rapid self/foreign discrimination

Science

Garvan医学研究所，2018





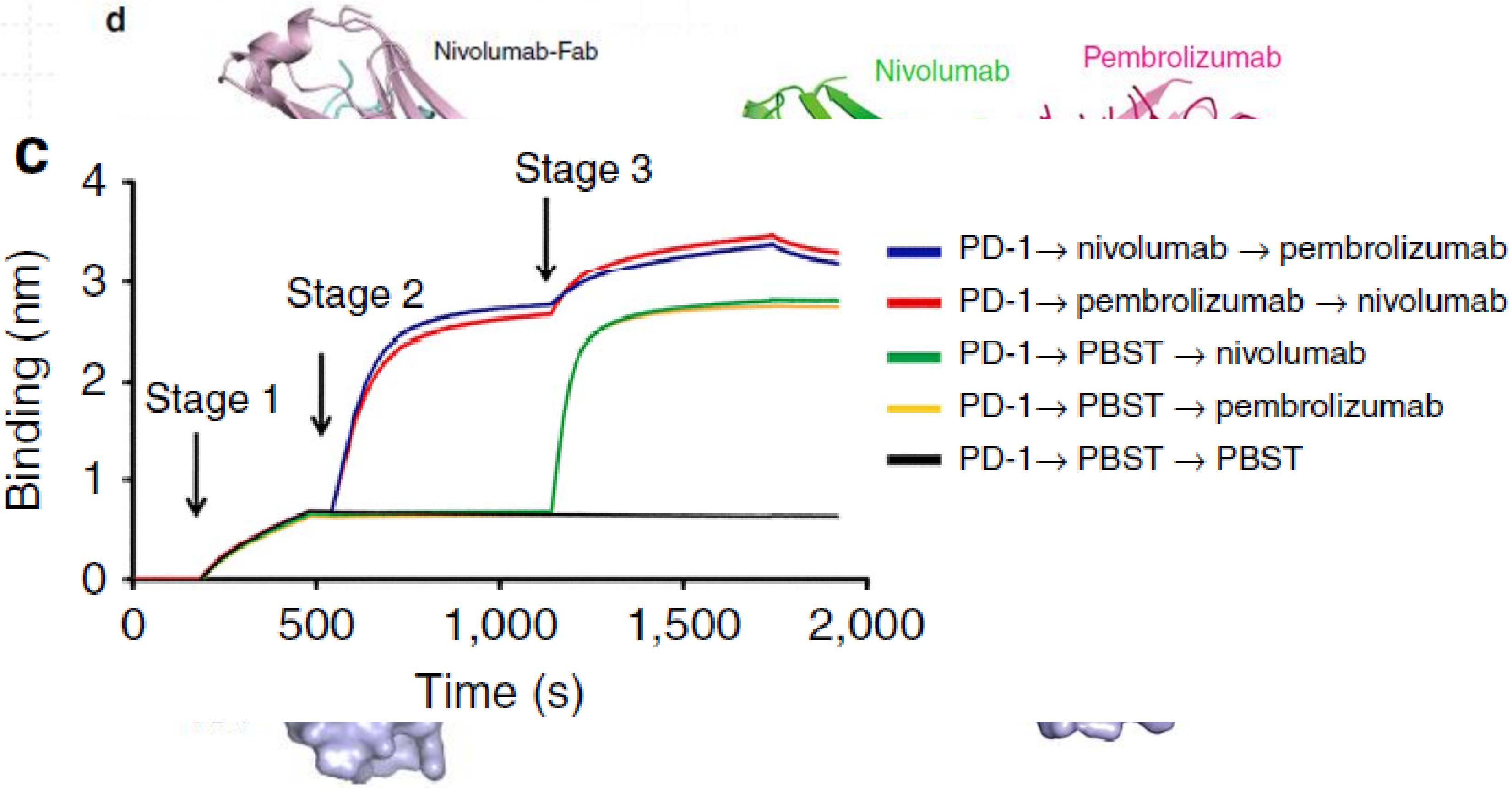
1. “坏抗体”自身免疫抗体，通常会被沉默；
2. 在感染外源抗原后，会经历一个快速的“救赎”，从“坏”变“好”的过程，经过三个氨基酸位点突变快速变成对抗外来入侵者的有力武器。
3. 通过晶体结构的结果以及亲和力的数据，从机制上阐述了免疫系统如何保护机体，‘坏抗体’将成为开发HIV和其他疾病疫苗的、非常有价值的资源。

PD-1抗体结合位点分析

An unexpected N-terminal loop in PD-1 dominates binding by nivolumab

Shuguang Tan[1,2,*], Hao Zhang[1,3,*], Yan Chai[2,*], Hao Song[4], Zhou Tong[1], Qihui Wang[1], Jianxun Qi[2], Gary Wong[2], Xiaodong Zhu[5], William J. Liu[6], Shan Gao[7], Zhongfu Wang[8], Yi Shi[2], Fuquan Yang[9], George F. Gao[2,4,6] & Jinghua Yan[1,2,3]

中科院微生物所, 2017

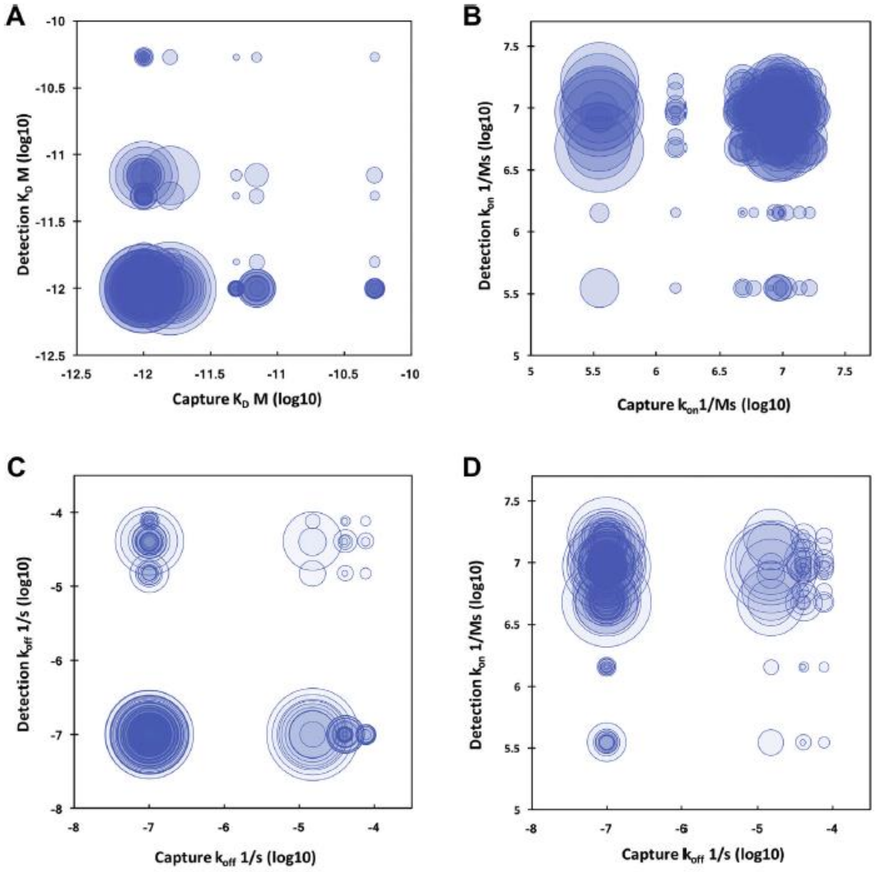


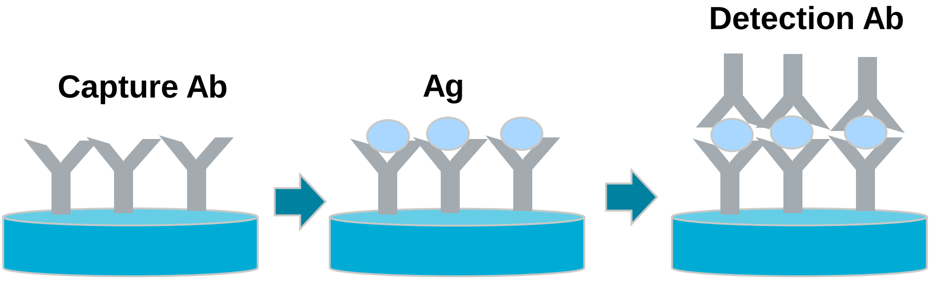
诊断抗体研究

Biolayer interferometry predicts ELISA performance of monoclonal antibody pairs for *Plasmodium falciparum* histidine-rich protein 2

C.F. Markwalter [a], I.K. Jang [b], R.A. Burton [b,1], G.J. Domingo [b], D.W. Wright [a,*]

[a] *Department of Chemistry, Vanderbilt University, Nashville, TN 37235, USA*
[b] *PATH, Seattle, WA 98121, USA*





Sandwich ELISA

- 捕获抗体：考虑慢解离slow koff
- 检测抗体：考虑快结合fast kon
- 两个抗体要综合选择
- BLI可以加速诊断抗体研发

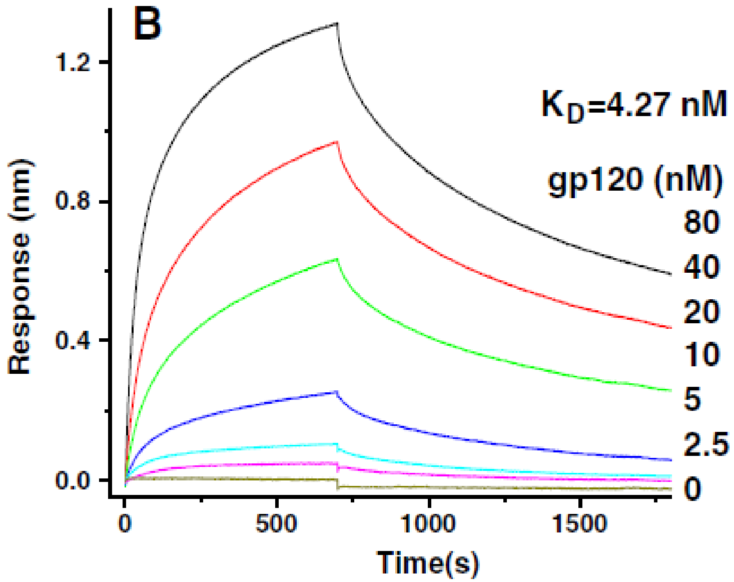
多糖与蛋白

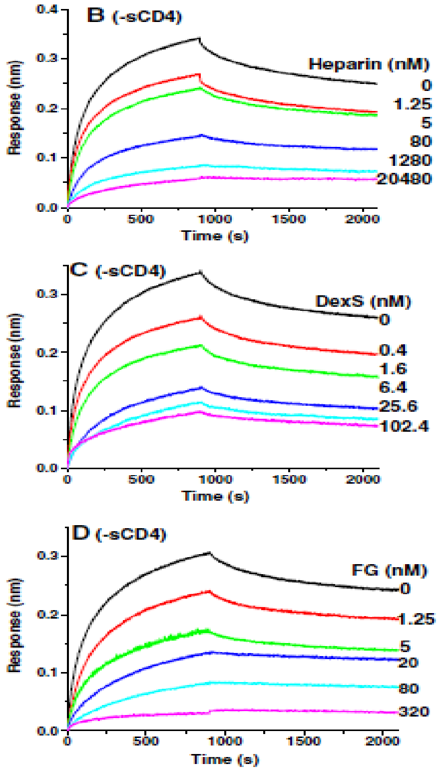
Anti-HIV-1 activity and structure–activity-relationship study of a fucosylated glycosaminoglycan from an echinoderm by targeting the conserved CD4 induced epitope

Wu Lian [a,b,1], Mingyi Wu [a,1], Ning Huang [c], Na Gao [a], Chuang Xiao [a,b], Zi Li [a], Zhigang Zhang [a], Yongtang Zheng [c,*], Wenlie Peng [b,*], Jinhua Zhao [a,*]

BBA General Subjects

中科院昆明植物所，2013





多糖2（IC50=1.1nM）

>多糖3（1.2nM）

>多糖1（10.1nM）

检测多糖1和gp120蛋白的结合

竞争法测试多糖2,3,4和gp120蛋白的结合能力

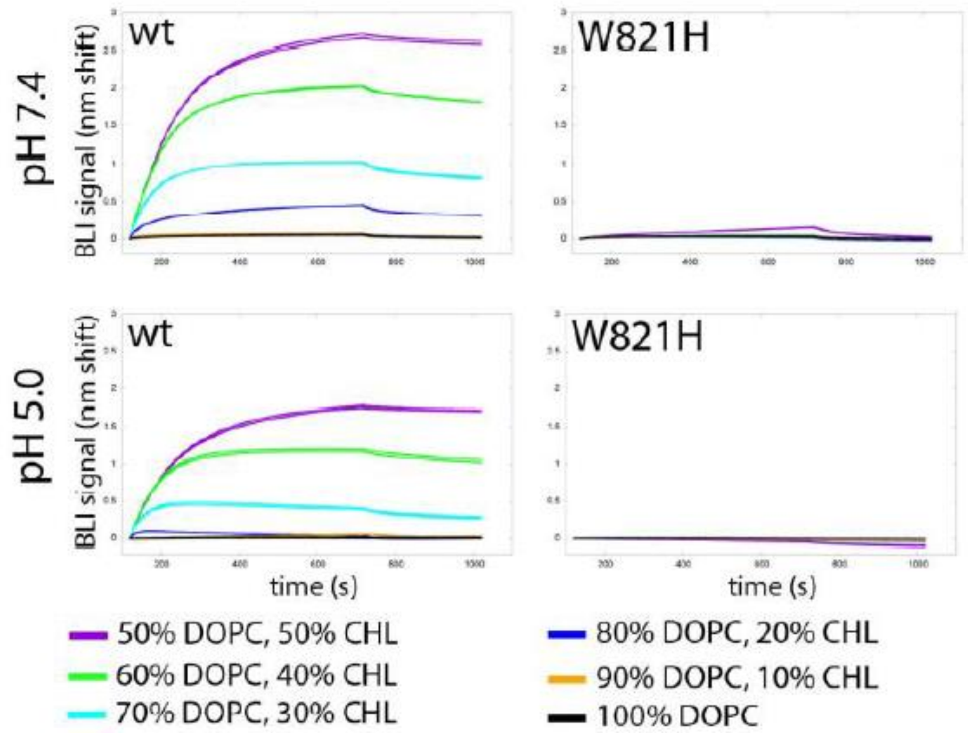
脂质体与蛋白

A glycerophospholipid-specific pocket in the RVFV class II fusion protein drives target membrane insertion

P. Guardado-Calvo, K. Atkovska, S. A. Jeffers, N. Grau, M. Backovic, J. Pérez-Vargas, S. M. de Boer, M. A. Tortorici, G. Pehau-Arnaudet, J. Lepault, P. England, P. J. Rottier, B. J. Bosch, J. S. Hub and F. A. Rey

Science

法国巴斯德所，2017



包膜蛋白不同pH下都可以与脂质体（细胞膜）结合，而突变的包膜蛋白则无法结合

本研究发现，裂谷热病毒的包膜蛋白插入到宿主细胞膜中融合形成一种包膜蛋白-脂质复合体结构。这种包膜蛋白具有一种特异性地识别细胞膜组分脂质亲水性头部的“口袋”。

重要的是，这种“识别口袋”在由节肢动物传播的其他病毒（如登革热病毒、寨卡病毒和基孔肯雅病毒）的包膜蛋白中发现。

这为抗病毒治疗的研究提供了启示。

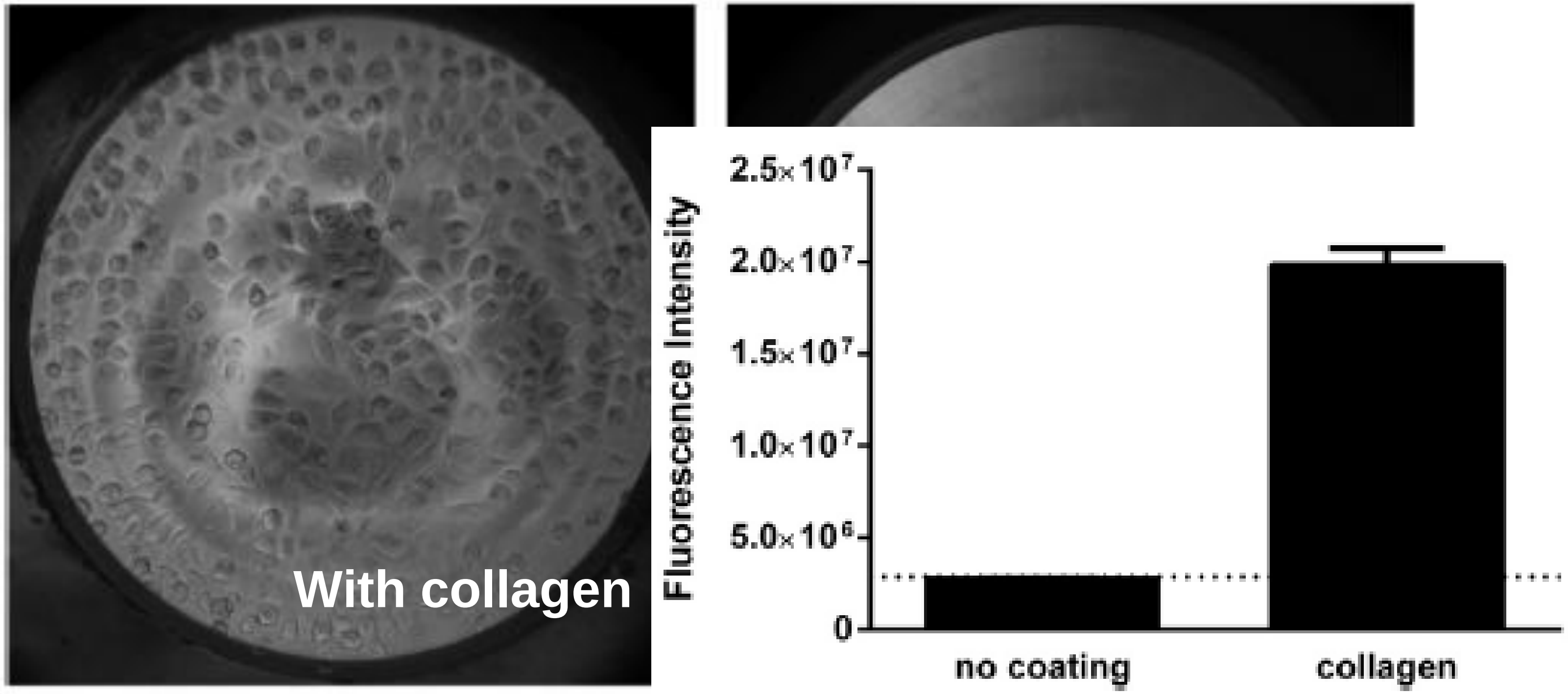
细胞互作研究

A novel label-free cell-based assay technology using biolayer interferometry

D. Verzijl [a], T. Riedl [a], P.W.H.I. Parren [a,b], A.F. Gerritsen [a,*]

[a] *Genmab, Yalelaan 60, Utrecht, 3584 CM, The Netherlands*
[b] *Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, Albinusdreef 2, Leiden, 2333 ZA, The Netherlands*

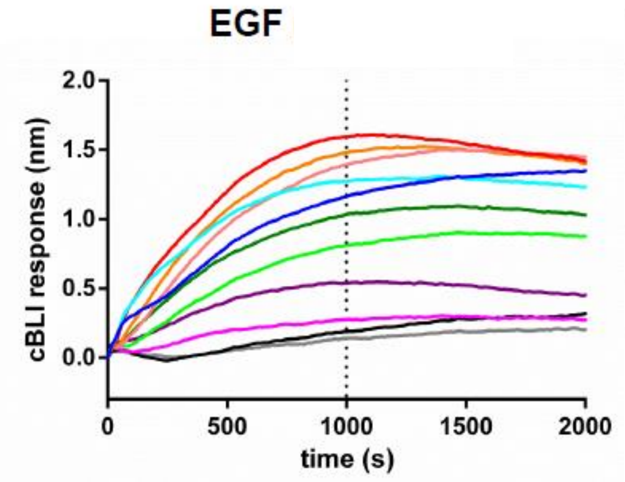
Genmab公司 2016

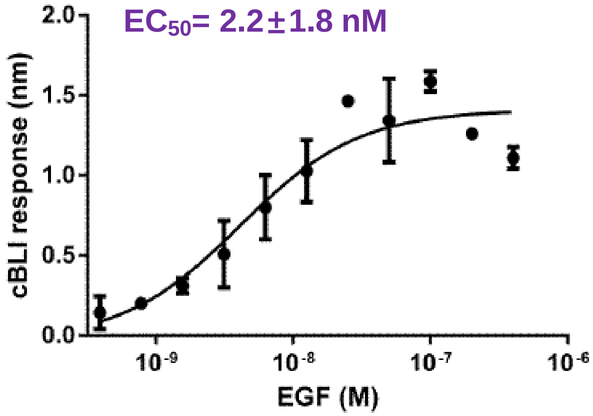


AR2G传感器结合Collagen，然后浸入Human A431细胞悬液中固化

荧光染色检测细胞活性，P值 < 0.0001

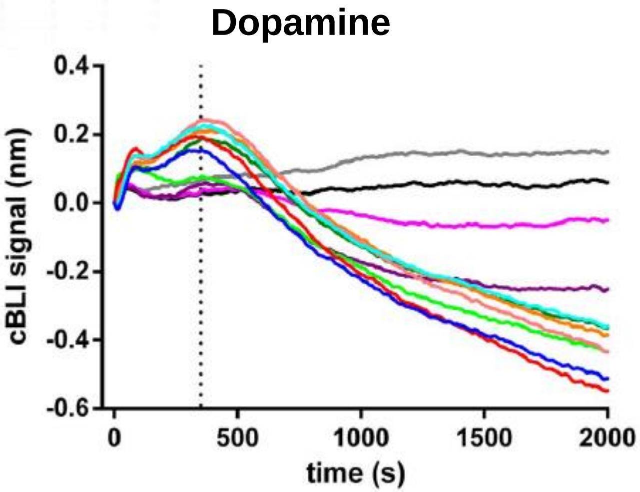
细胞互作研究

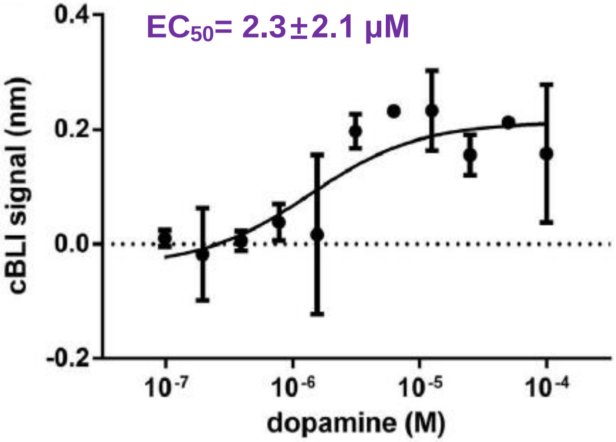




Human A431细胞表面表达EGFR，
其配体蛋白为EGF（6 kDa）

与不同浓度的EGF进行检测，发现
response信号与浓度呈正相关





Human A431细胞内源性表达β2-
adrenoceptor，可被dopamine
（153Da）激活

与不同浓度的dopamine进行检测，
发现response信号与浓度呈正相关

CAR-T应用

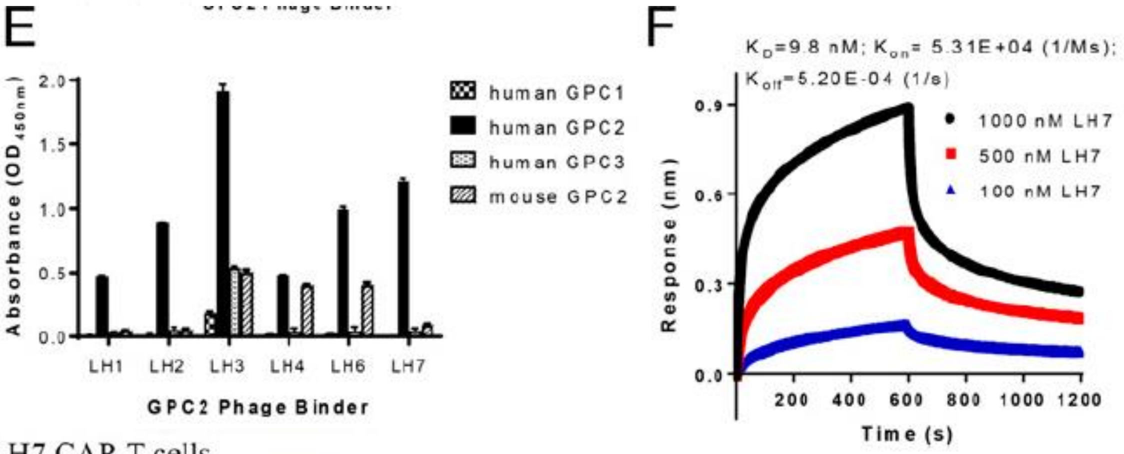
Therapeutically targeting glypican-2 via single-domain antibody-based chimeric antigen receptors and immunotoxins in neuroblastoma

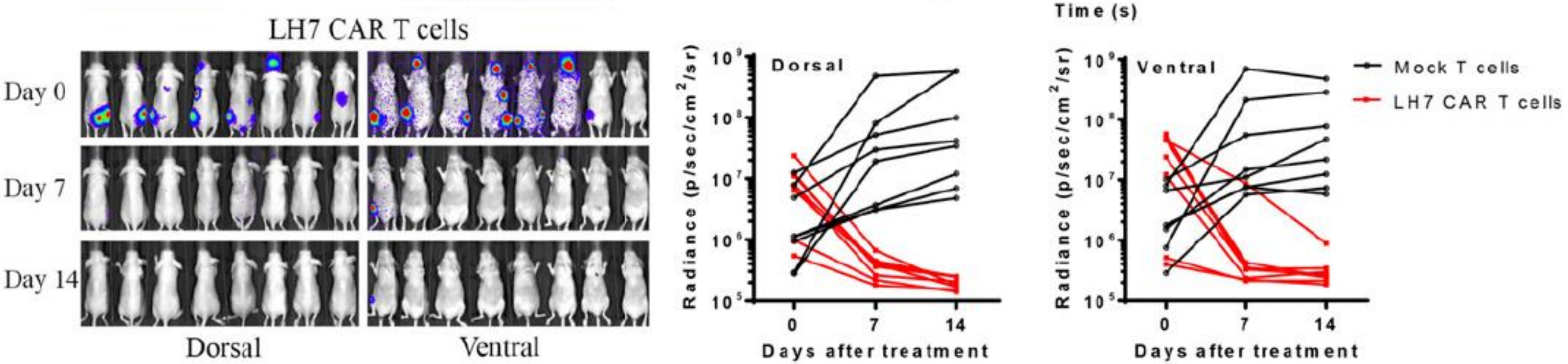
PNAS

PNAS，2017

Nan Li[a], Haiying Fu[a,b], Stephen M. Hewitt[c], Dimiter S. Dimitrov[d], and Mitchell Ho[a,1]

[a]Laboratory of Molecular Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; [b]Department of Immunology, Norman Bethune College of Medicine, Jilin University, Changchun 130021, China; [c]Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; and [d]Cancer and Inflammation Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Frederick, MD 21702



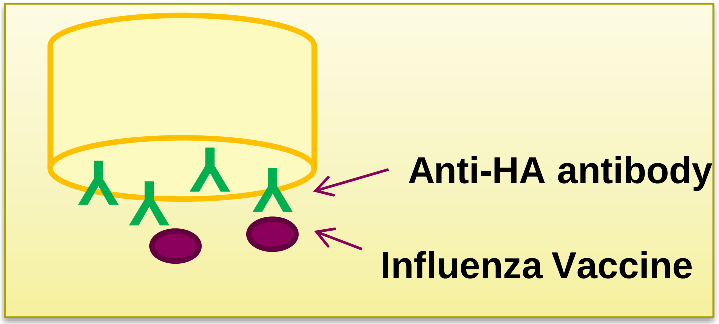


定量检测

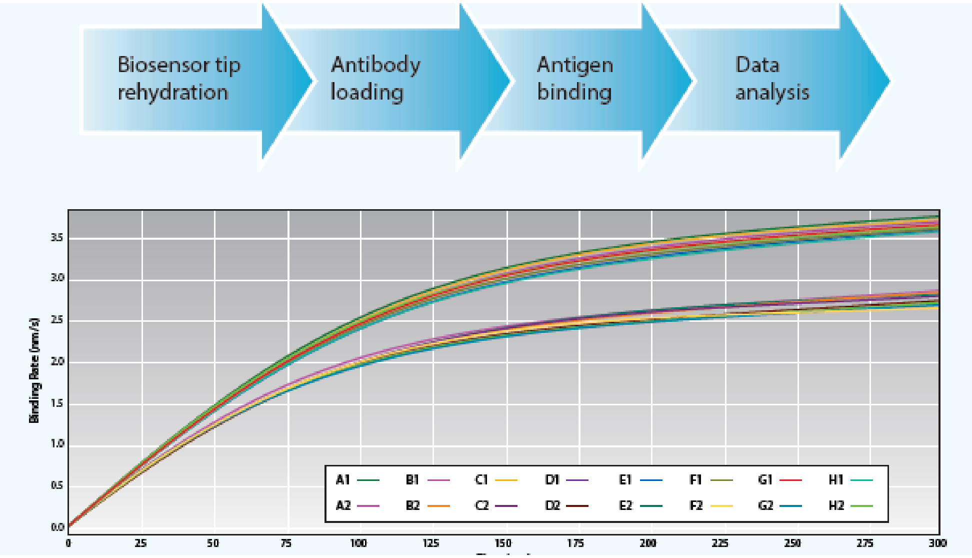
流感疫苗定量

传统方法—单向免疫扩散（SRID）

优点	缺点
□ 金标准	□ 用时长（2 ~ 3天）
□ 使用简便	□ 检测通量较低
□ 检测成本较低	□ 动态范围较窄
	□ 检测准确度、精确度不理想

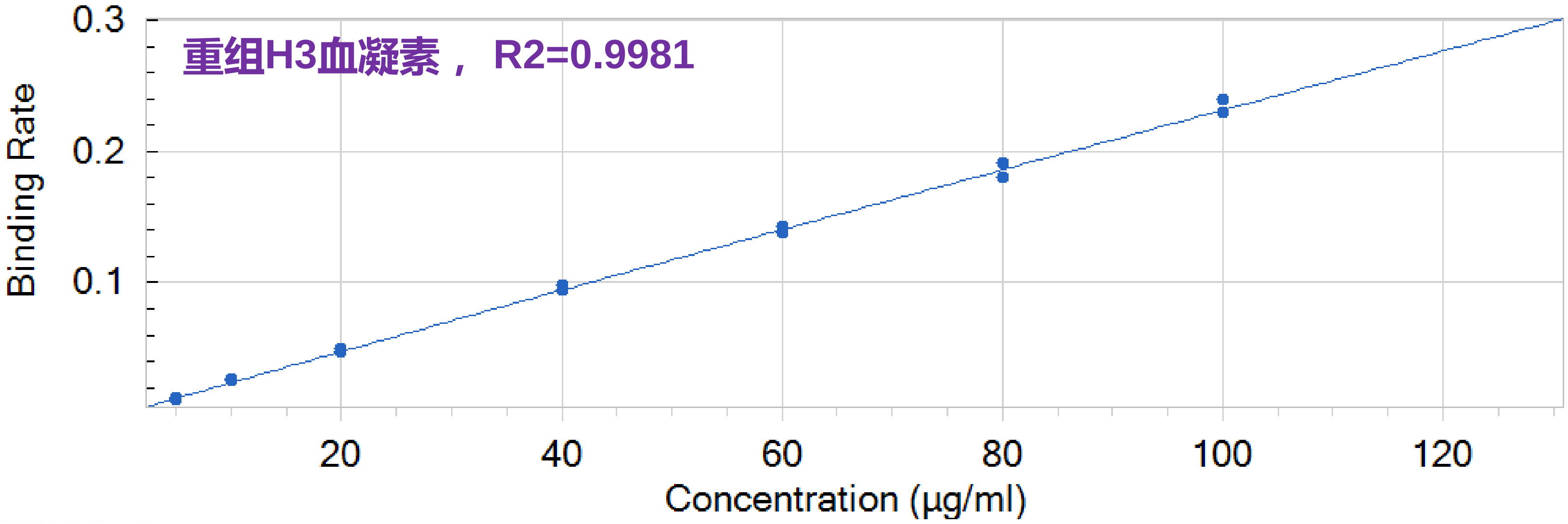


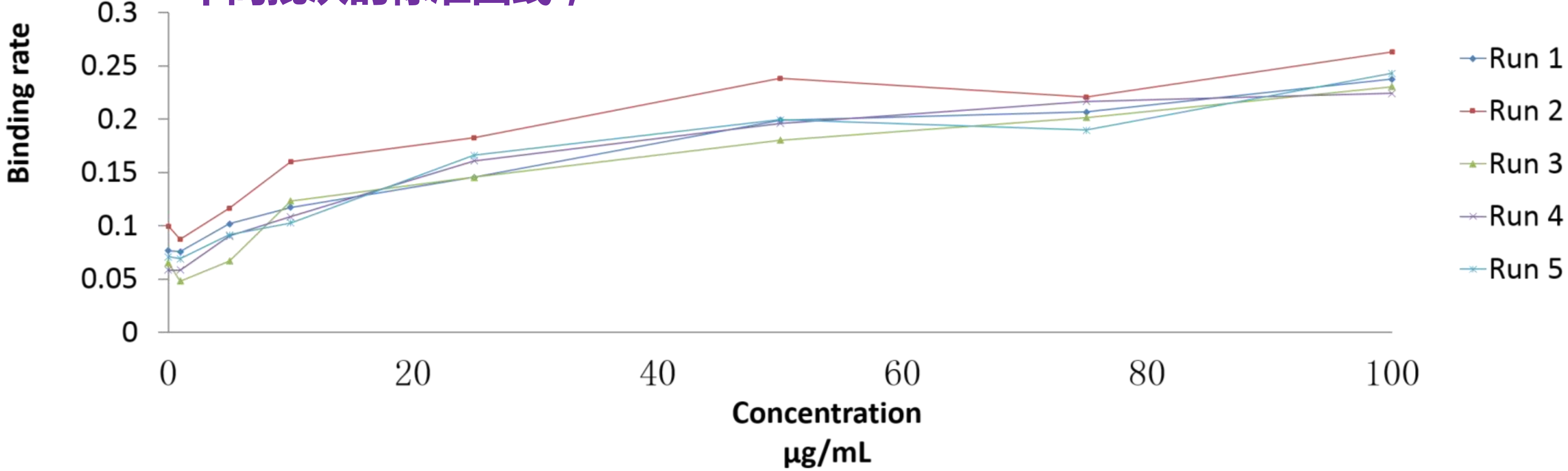
实验设计



Loading的准确性（两种不同抗体）

流感疫苗定量


重组H3血凝素， R2=0.9981

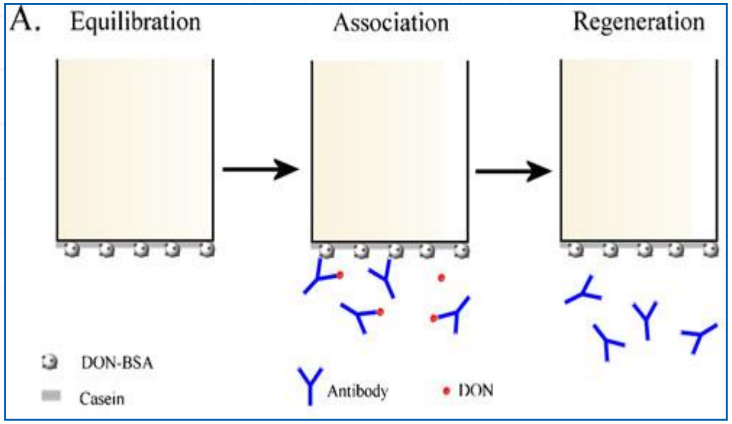

不同批次的标准曲线，CV < 7%

小麦呕吐毒素检测

Detection of deoxynivalenol using biolayer interferometry

Chris M. Maragos

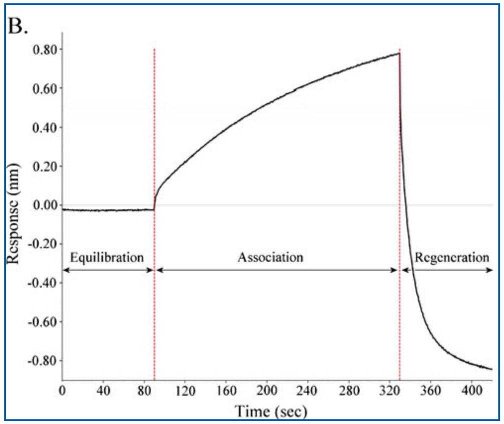
Mycotox Res (2011) 27:157–165

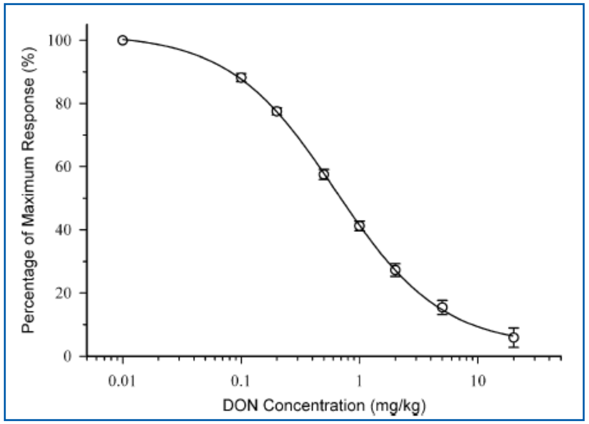


Deoxynivalenol（DON）
呕吐毒素，又称脱氧雪腐镰刀菌烯醇，真菌毒素，296Da，常见于霉变的谷物

检测方法---竞争法

1. DON-BSA固定在APS传感器上
2. 将过量的抗体和不同浓度的DON混合孵育
3. 传感器检测信号，建立标准曲线
4. 检测未知浓度样品
5. 再生





检出限：0.1mg/Kg

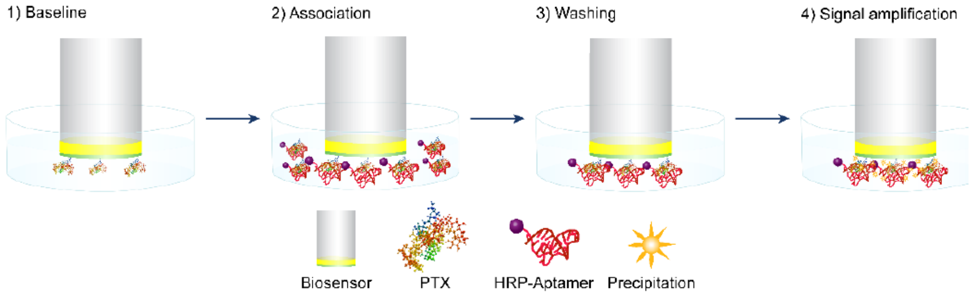
回收率：101.4%

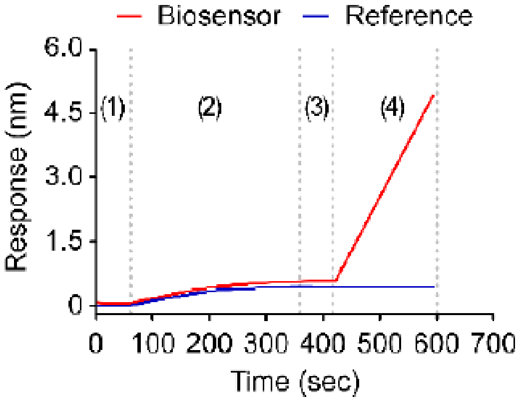
RSD：13.2%

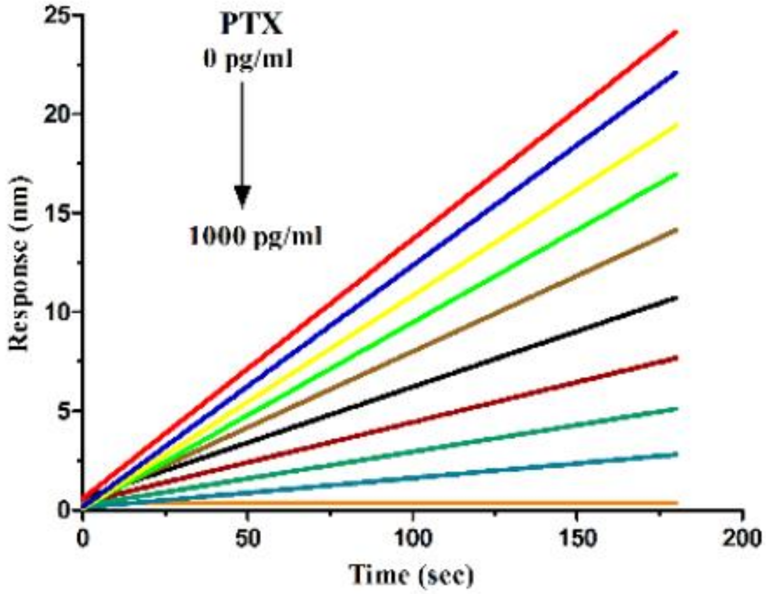
适配子定量海葵毒素

Enzyme-linked, aptamer-based, competitive biolayer interferometry biosensor for palytoxin

第二军医大学，2016







回收率100.27%~108.24%，说明贝类海鲜的提取物基质对检测没有明显影响。

变异系数2.27%~6.76%，重复性非常好。

检测极限为0.04pg/ml。

内 容

简介

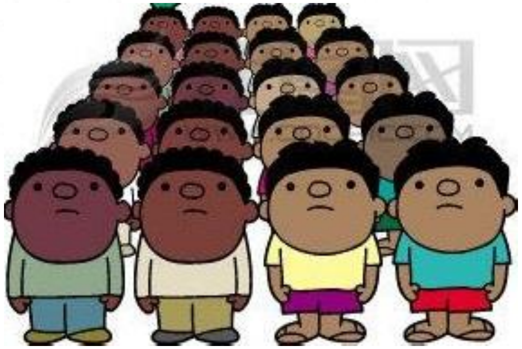
原理

应用

系统特点

特点一：检测速度最快

OCTET



- 8通道平行检测
- 通量高
- 快速获得实验结果
- 数据准确性高，一致性好

SPR

- 单通道串联检测
- 通量较低
- 实验耗时长
- 一致性依赖再生条件

特点二：操作最简便

OCTET

SPR

偶联pH

样品浓度

缓冲液
条件

再生条件

- 简明易用，适合普通使用者
- 直观的实验设计流程
- 同时优化多种条件，灵活快速（1h）

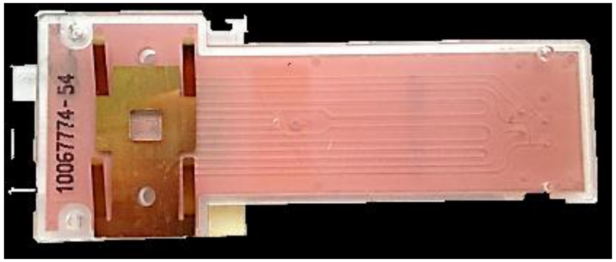
- 操作复杂，需要专业使用者
- 多数实验需要编辑程序
- 复杂的实验条件优化，耗时冗长（6-10h）

特点三：维护最简单

OCTET

- **无流路**阻塞之忧
- 样品无需离心脱气
- 可以检测**粗样品**，应用广
- 无惧有机溶剂和甘油

SPR



- 微流路系统（IFC）为易损耗件
- 自然老化，需**定期更换**（每6-10个月）
- 更换费用昂贵（**约$7000**)
- 无法检测粗样品和易沉淀样品，会**堵塞IFC**
- 不能使用较高浓度的有机溶剂和甘油

特点四：实验及维护成本最低

OCTET

SPR

时间成本

< 1小时

6-12小时

使用成本

传感器（$5-7/个）

传感芯片($250-1200/片),还有试剂盒…

维护成本

无易损件

更换流路，USD 7000/每年

总结

- Octet 系统灵敏度高、通用性强、适合复杂样品
- 实验步骤设计简便，通量高，检测速度快
- 软件操作与分析易学易用，快速入门
- 耗材使用、硬件维护成本很低，用户无负担
- 产品技术成熟，中国用户文献量逐年递增
- 强大的技术支持团队，提供一流的售后服务

又快！又准！又易用！又省成本！

Thank You!


octet HTX
WARNING: Keep hands clear of moving parts.
PALL FortéBio®