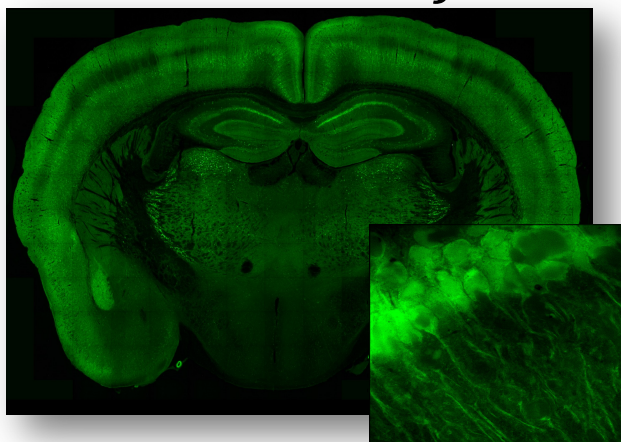
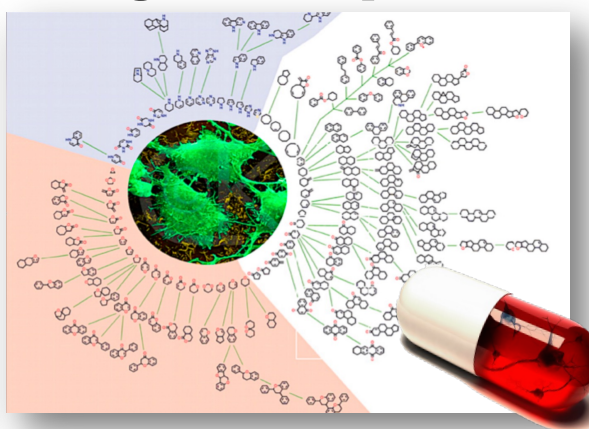


Chemical Biology Innovations in **Brain Drug Discovery**

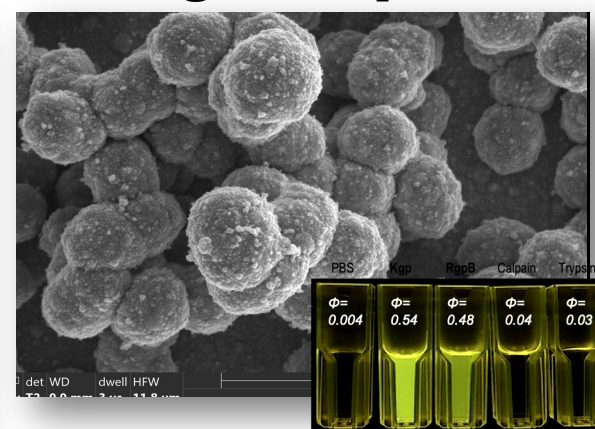
Innovative Platform



Drug Development



Theragnosis probes

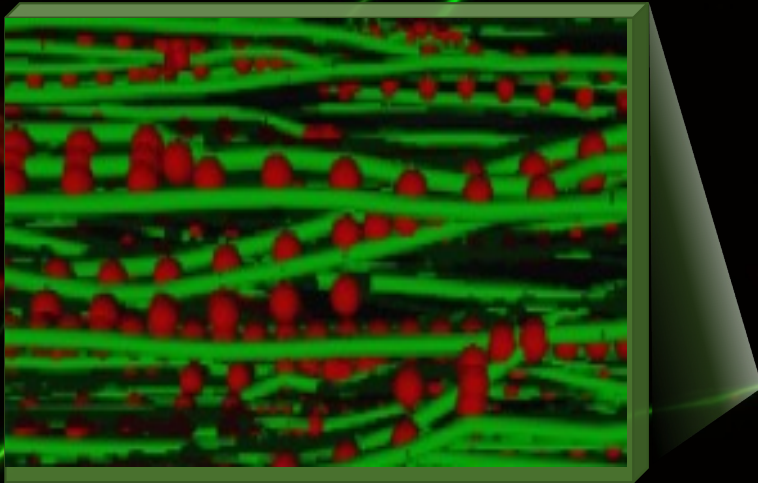


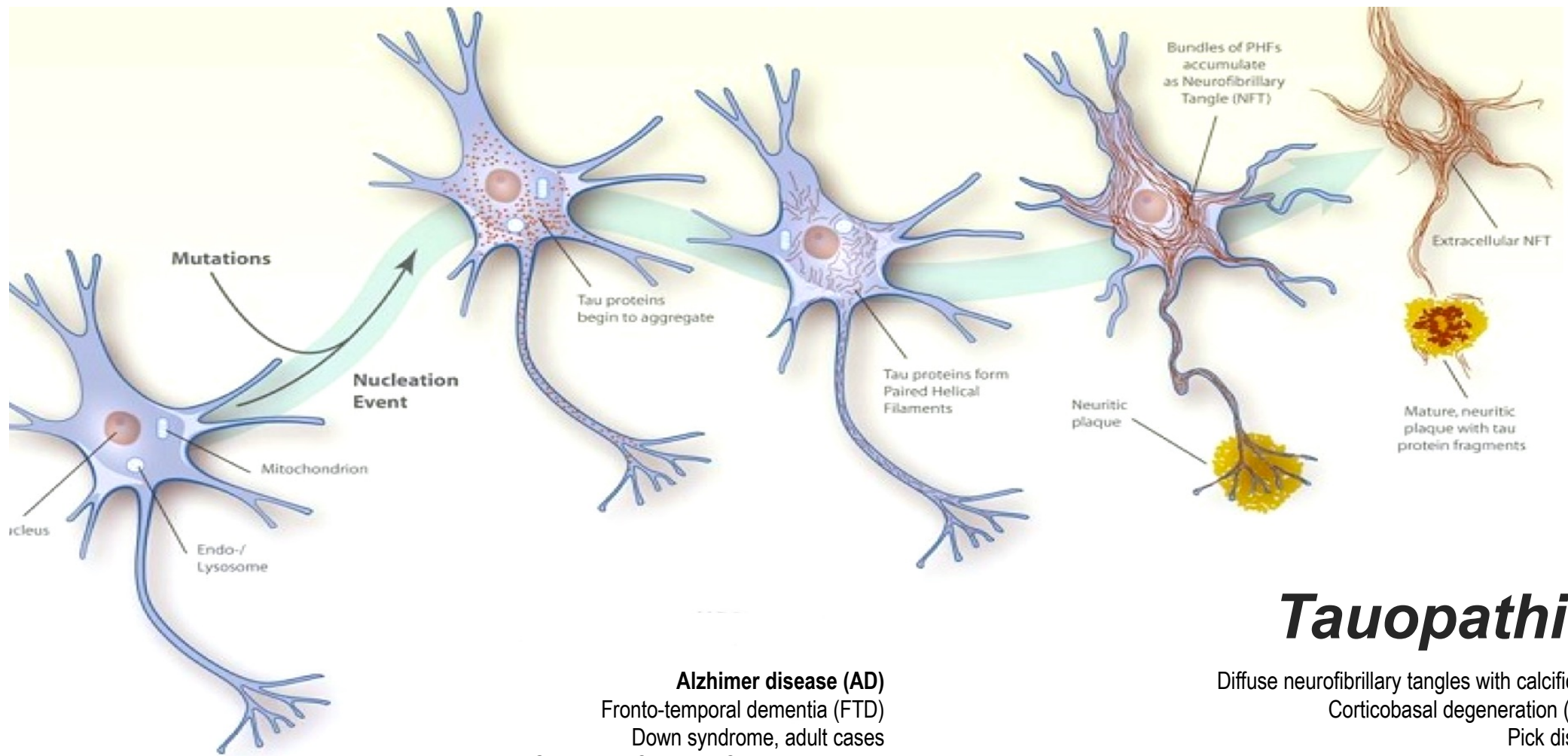
KIST Brain Science Institute

Yun Kyung Kim

① Tau protein is

microtubule stabilizing protein in neuron



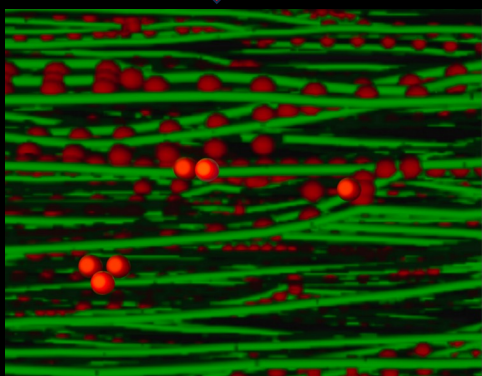
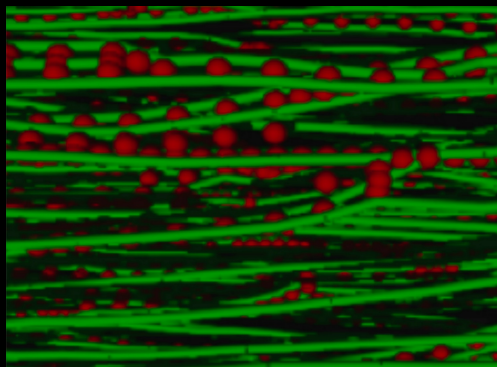


Tauopathies

Alzheimer disease (AD)
 Fronto-temporal dementia (FTD)
 Down syndrome, adult cases
 Gerstmann-Sträussler-Scheinker disease
 Hallervorden-Spatz disease
 Inclusion body myositis
 Multiple system atrophy
 Niemann-Pick disease type C (NPC)

Diffuse neurofibrillary tangles with calcification
 Corticobasal degeneration (CBD)
 Pick disease
 Progressive supranuclear palsy (PSP)
 Progressive supranuclear gliosis (PSG)
 Tangle only dementia
 Amyotrophic lateral sclerosis/ parkinsonism–dementia complex (ALS-FTDP)
 Frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17)

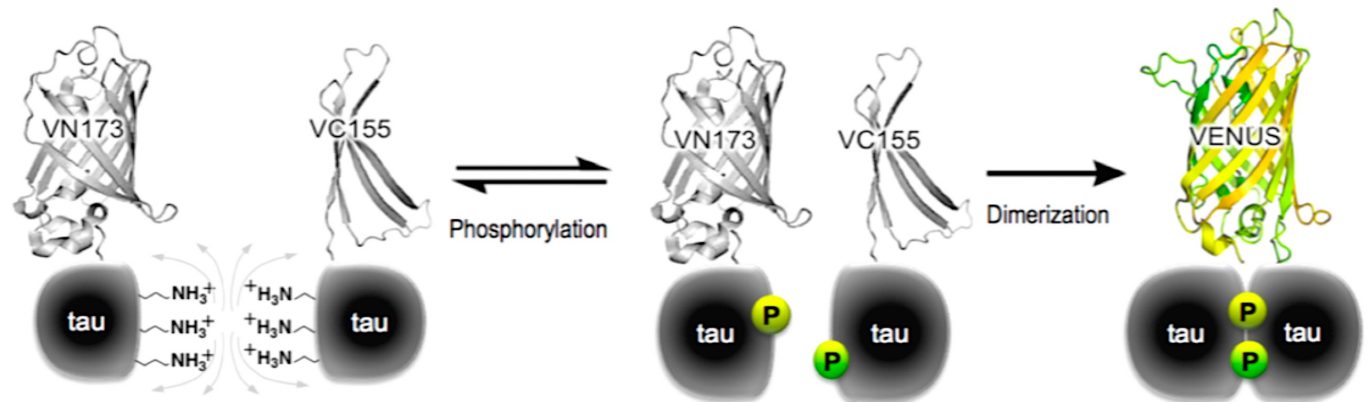
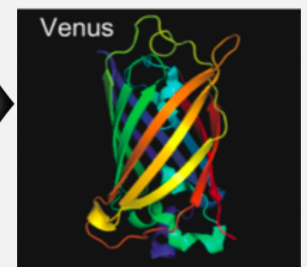
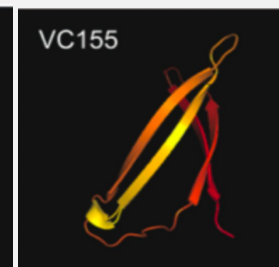
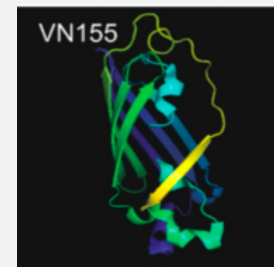
To detect *tau oligomers*



① **Tau-BiFC** as a fluorescence Turn-ON sensor *Lighting-up tau-tau interactions in living cells*

Bimolecular
Fluorescence
Complementation

BioTechniques, 2010, 49, 793

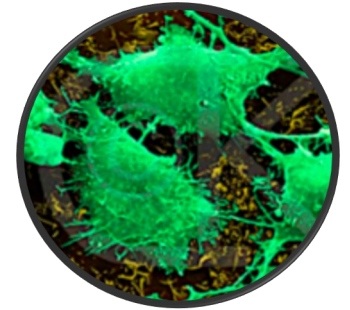
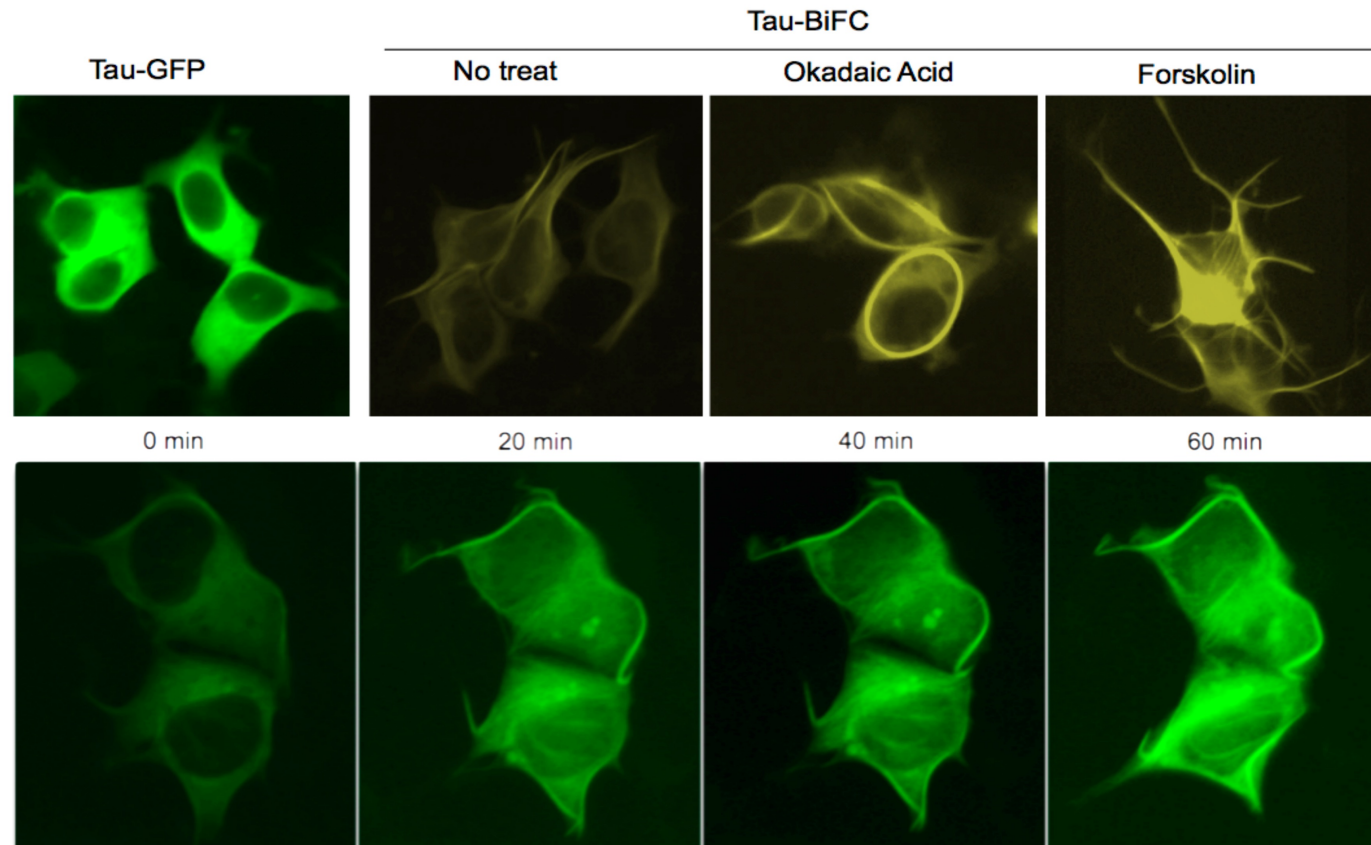


PLOS ONE, 2013, 8 (12):e81682

Patent: PCT/KR2014/001214/ 10-154645

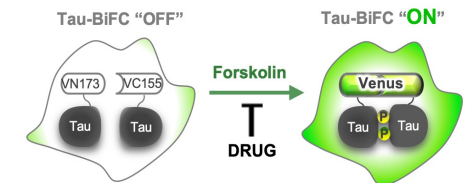
② *Tau-BiFC cell model*

Spatial & Temporal resolution of tau aggregation

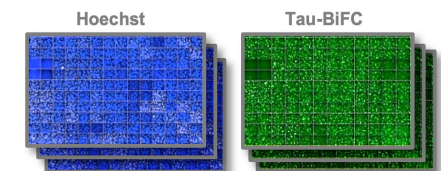


HTS-drug screening

- Day 1 ① Tau-BiFC cell plating on 384 well plates
- Day 2 ② Co-treatment of compound library with Forskolin
- ③ 'Turn-ON' Tau-BiFC by Forskolin (46 h)

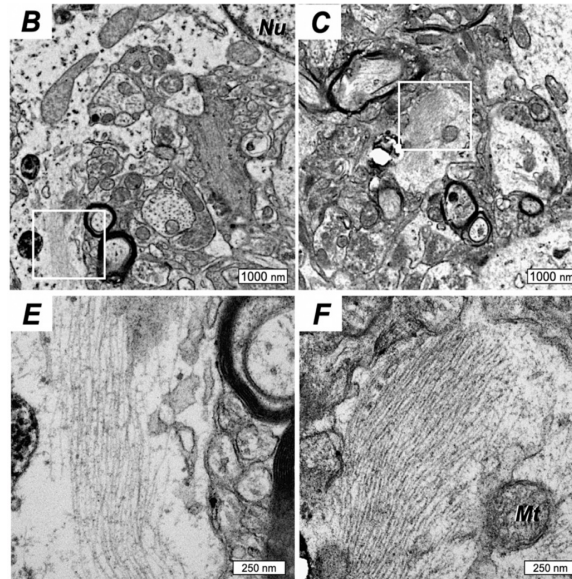
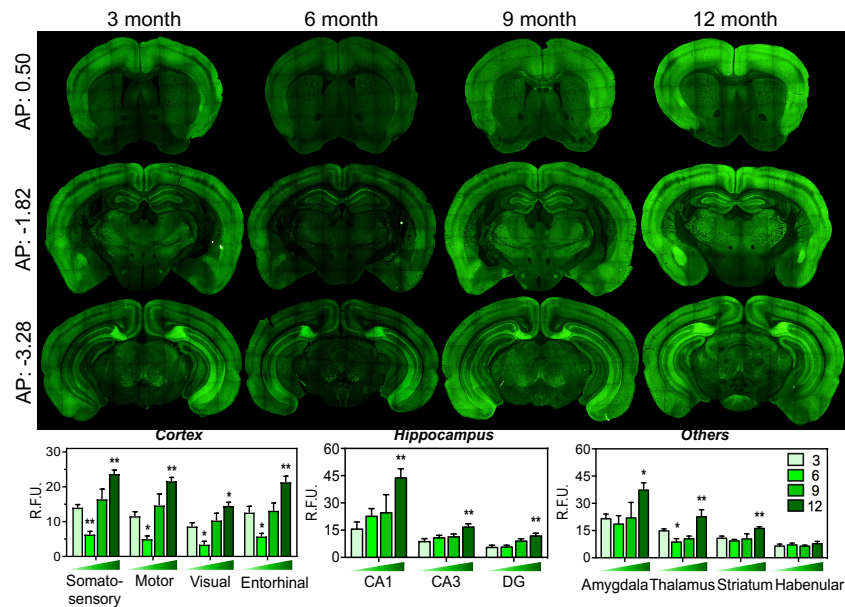
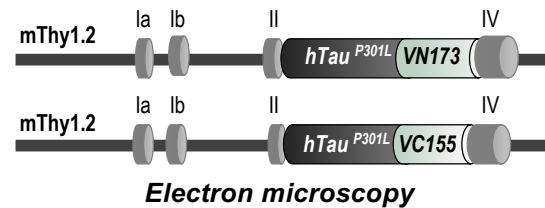
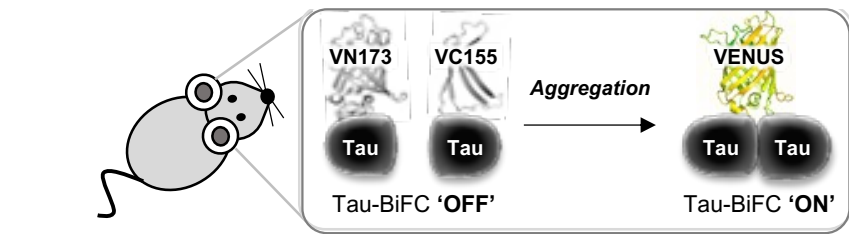


- Day 4 ④ Nuclei stain with Hoechst (2 h)
- ⑤ Image acquisition & analysis

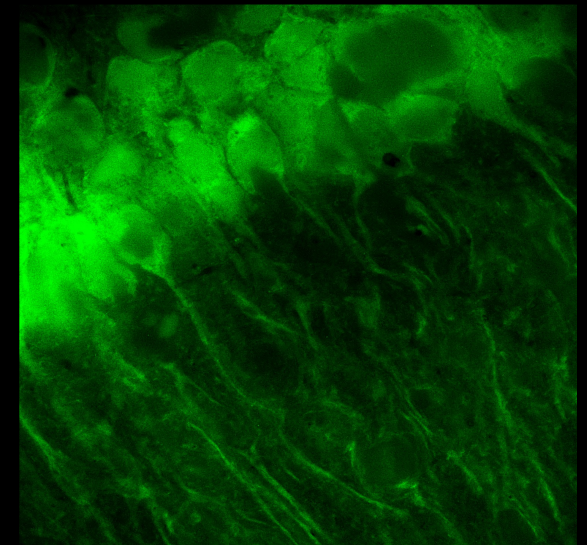


Patent: KR (10-1721920) Scientific report 2015, **Nature Comm 2014**, *IJMS* 2015, **Nature Comm 2016**, Scientific report 2017, **EMM 2018**, *IJMS* 2018, *IJMS* 2019, Scientific report 2019, et al.

③ *Tau (2N4R)-BiFC mouse model*



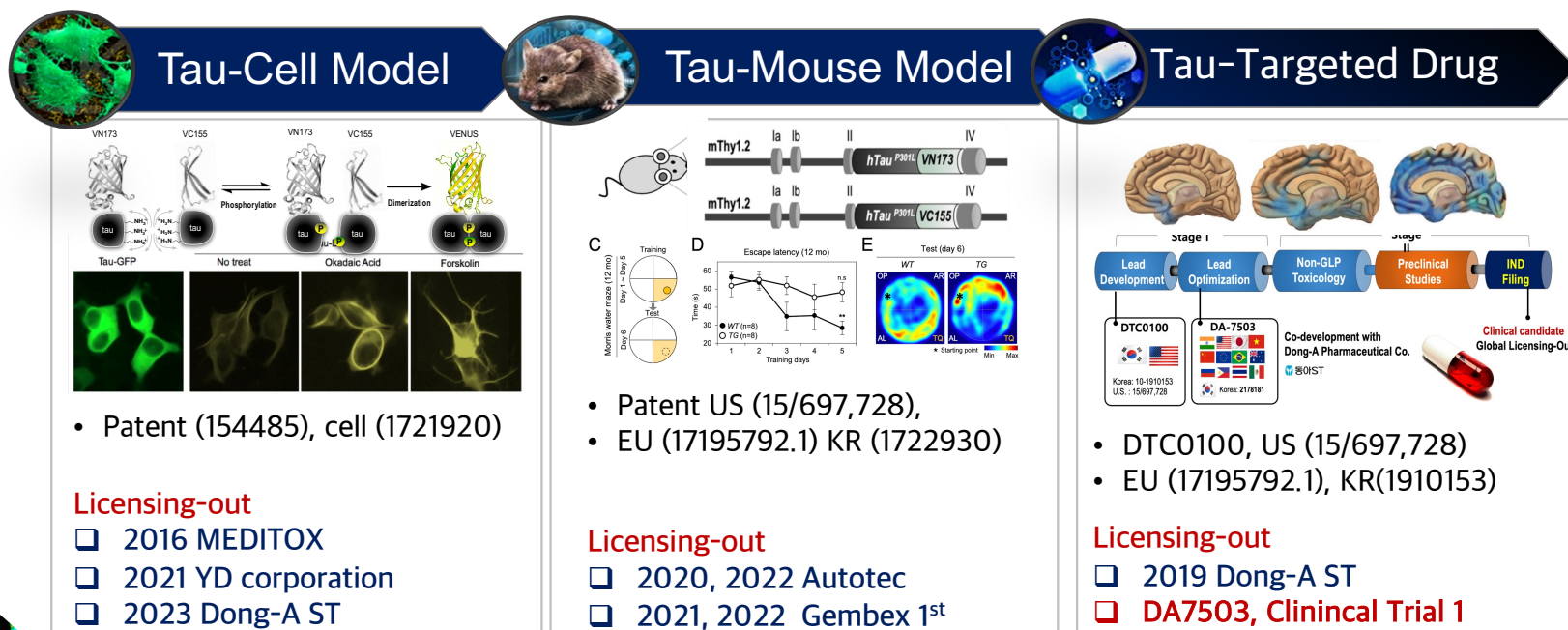
*Visualization of
Tau oligomerization
in the brain*

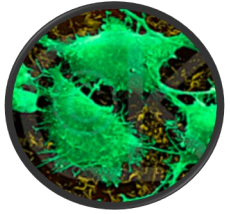


Patent: US (15/697,728) EU (17195792.1) KR(10-1722930) Progress in neurobiology, 2020

Bioactive Materials 2021, J. Alzheimer's Disease 2021, J. Neuroscience 2021, Nature Comm 2021, Biosensors and Bioelectronics 2021, Sensors and actuator B 2021, Nature Comm 2022,

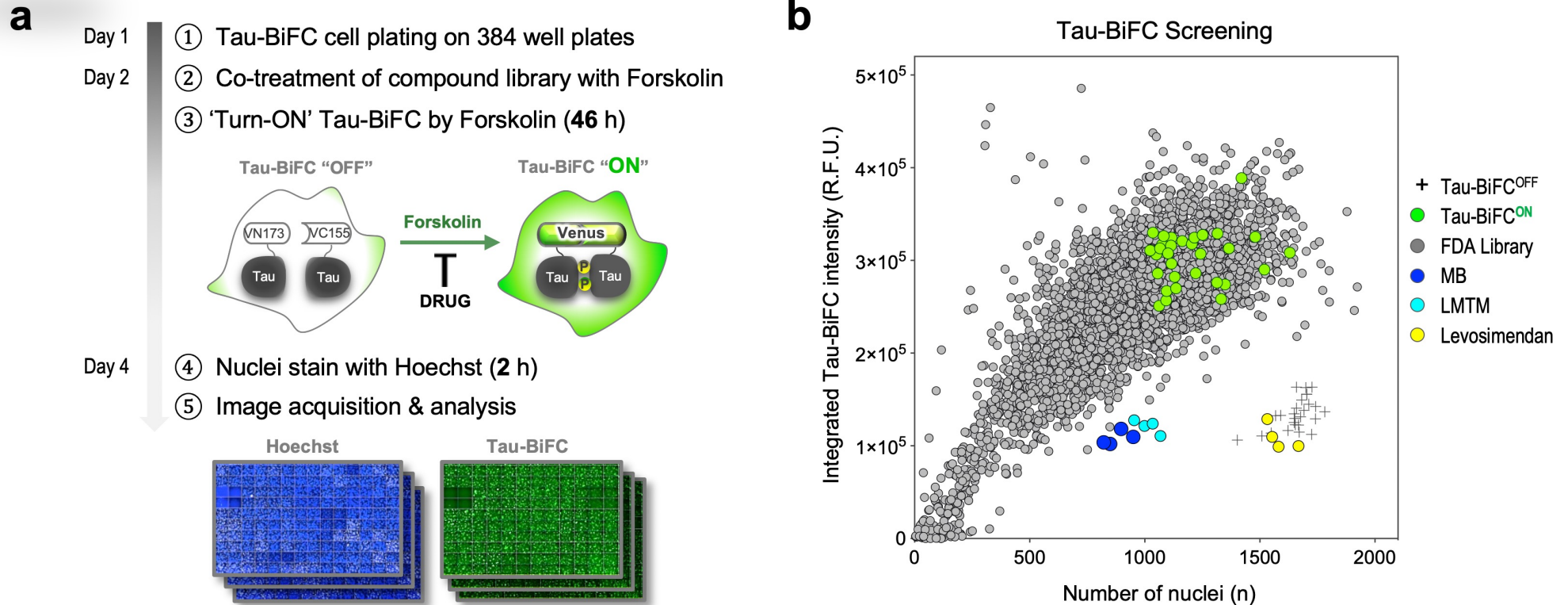
KIST Tau-Targeted Research Platform and Drug Development





Hit discovery

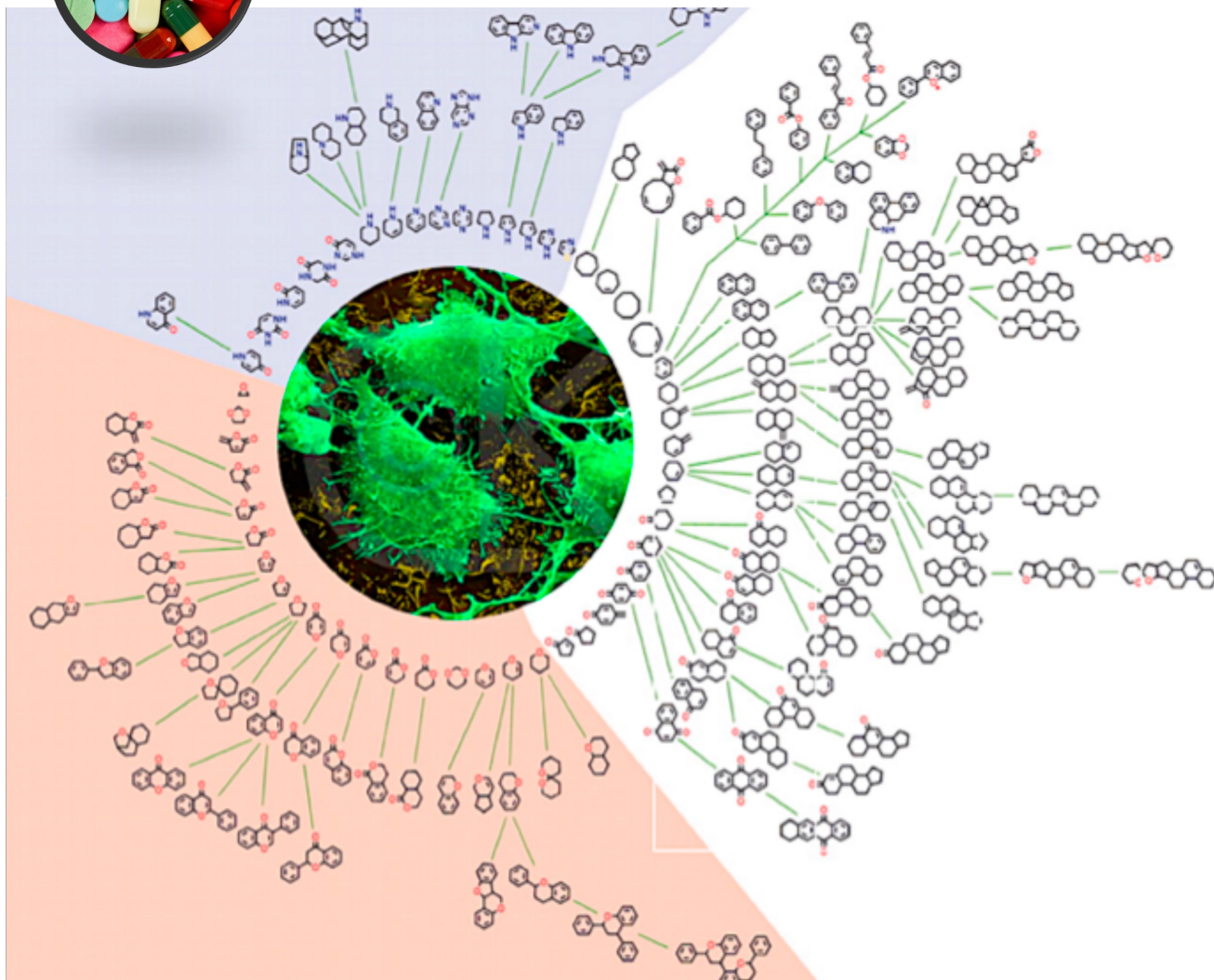
Identification of a new scaffold targeting tau-4R



Exp.Mol.Med. 2023

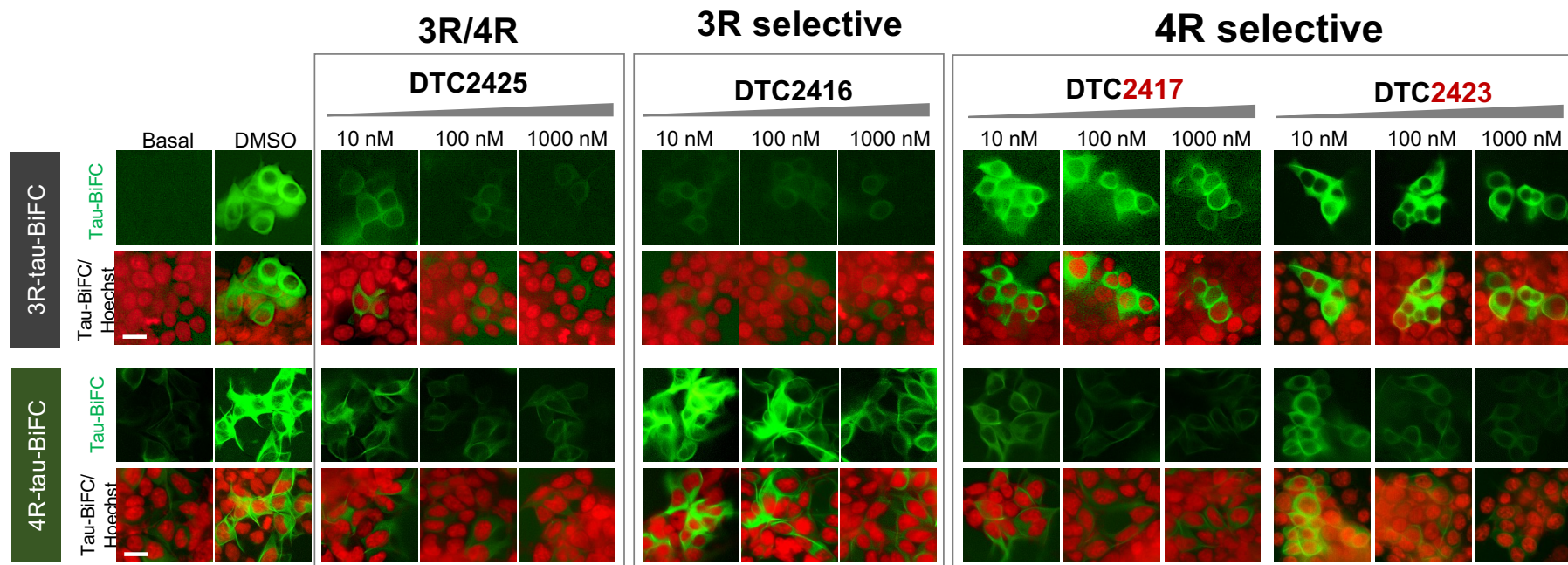


3R-4R tau Optimization



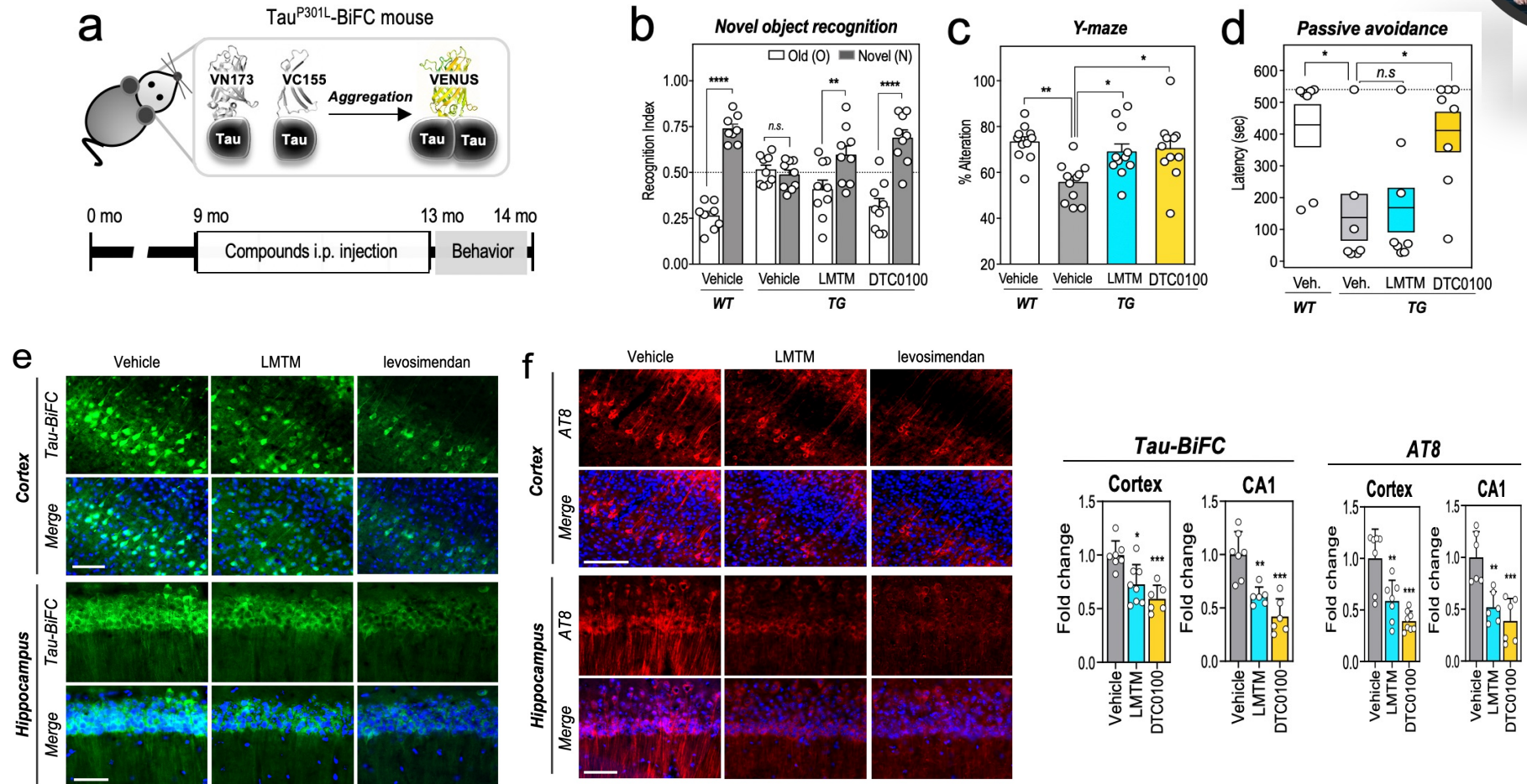
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1339	134				

3R/4R Tau–BiFC assay identified 3R/4R selective compounds



IC ₅₀		LMTM	DTC 2425	DTC 2416	DTC 2417	DTC 2423
Cell	4R-Tau-BiFC	2,210 nM	35 nM	126 nM	51 nM	88 nM
	3R-Tau-BiFC	3,500 nM	16 nM	4 nM	826 nM	757 nM
GI ₅₀		6 μM	78 μM	90 μM	121 μM	105 μM
In vitro	TauK18	1.80 μM	1.21 μM	-	0.27 μM	0.15 μM
	TauK19	-	1.69 μM	-	0.62 μM	0.71 μM
	Aβ 42	0.13 μM	22.5 μM	-	16.6 μM	15.1 μM

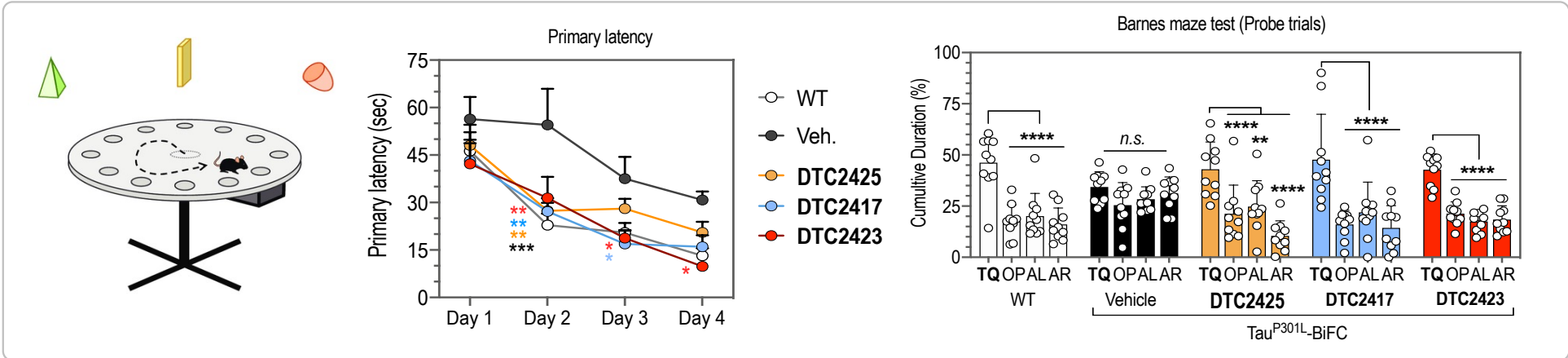
In vivo efficacy: Tau-BiFC mice



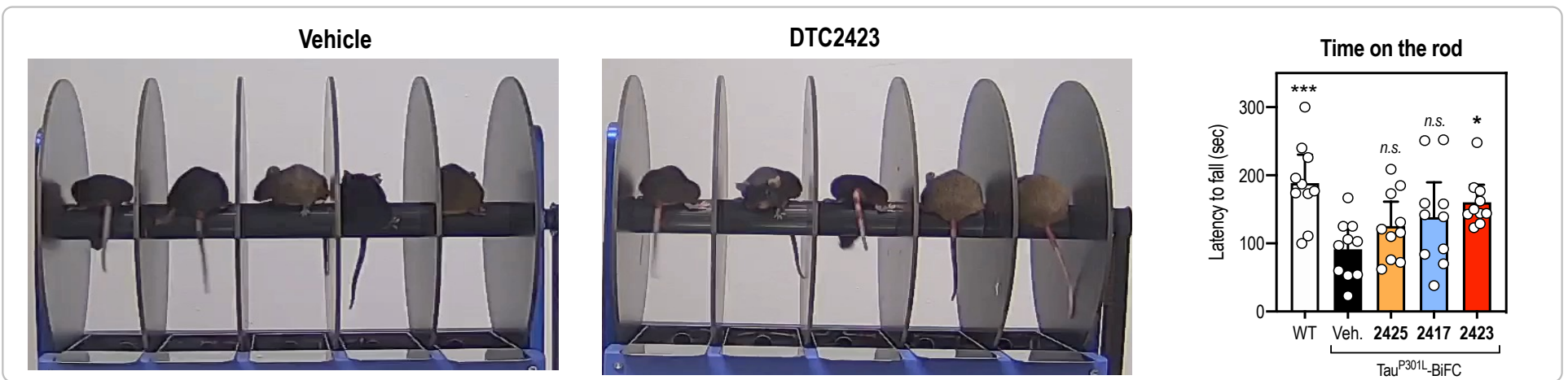
In vivo efficacy

Leads prevented cognitive and motor dysfunction in Tau-BiFC mice

④ Barnes-maze test: learning & memory



⑤ Rota-rod test: motor

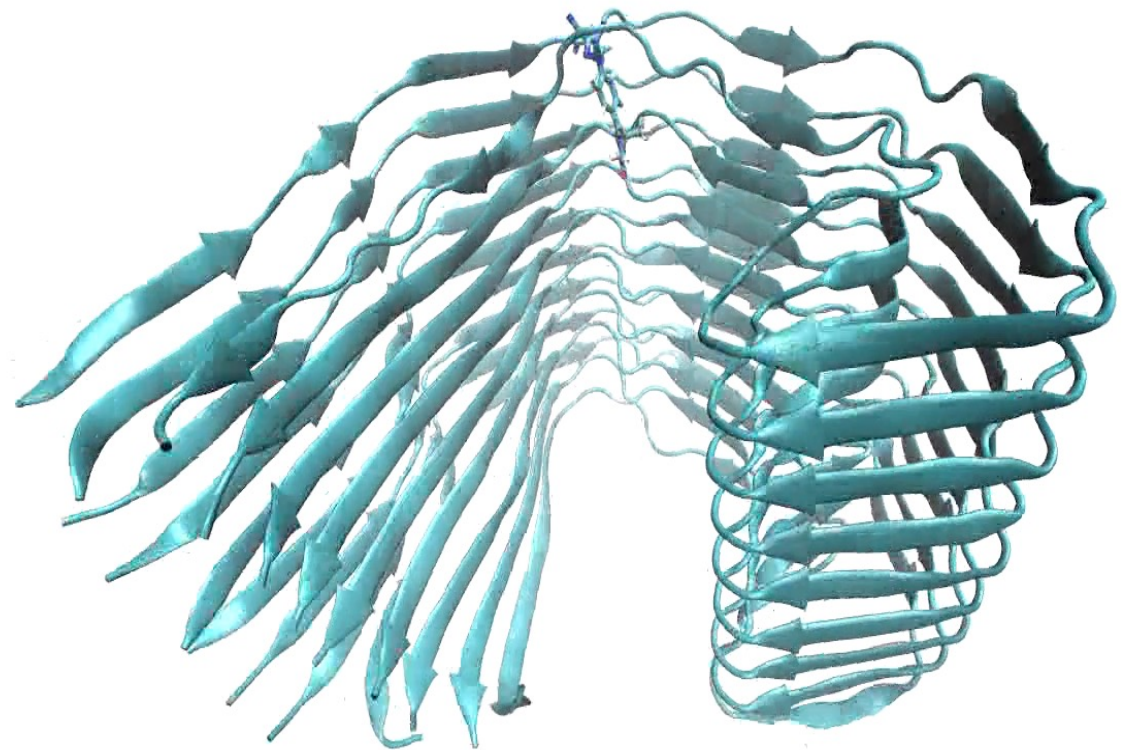
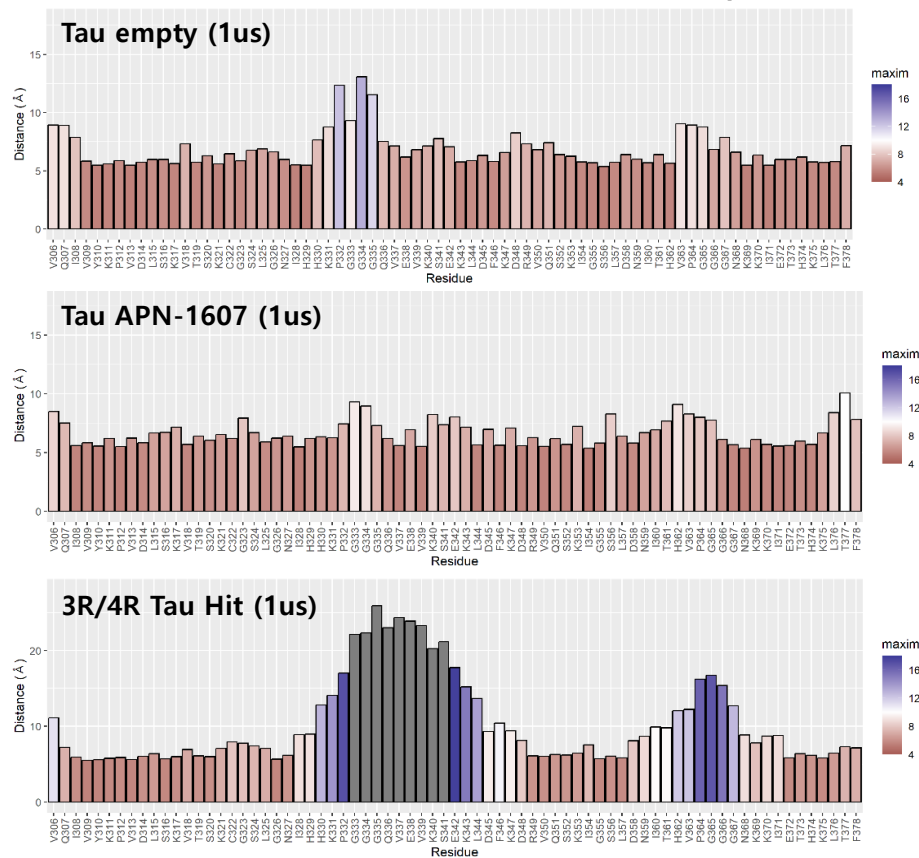


Covalent binding disrupts tau fibril structure during extended MD simulation

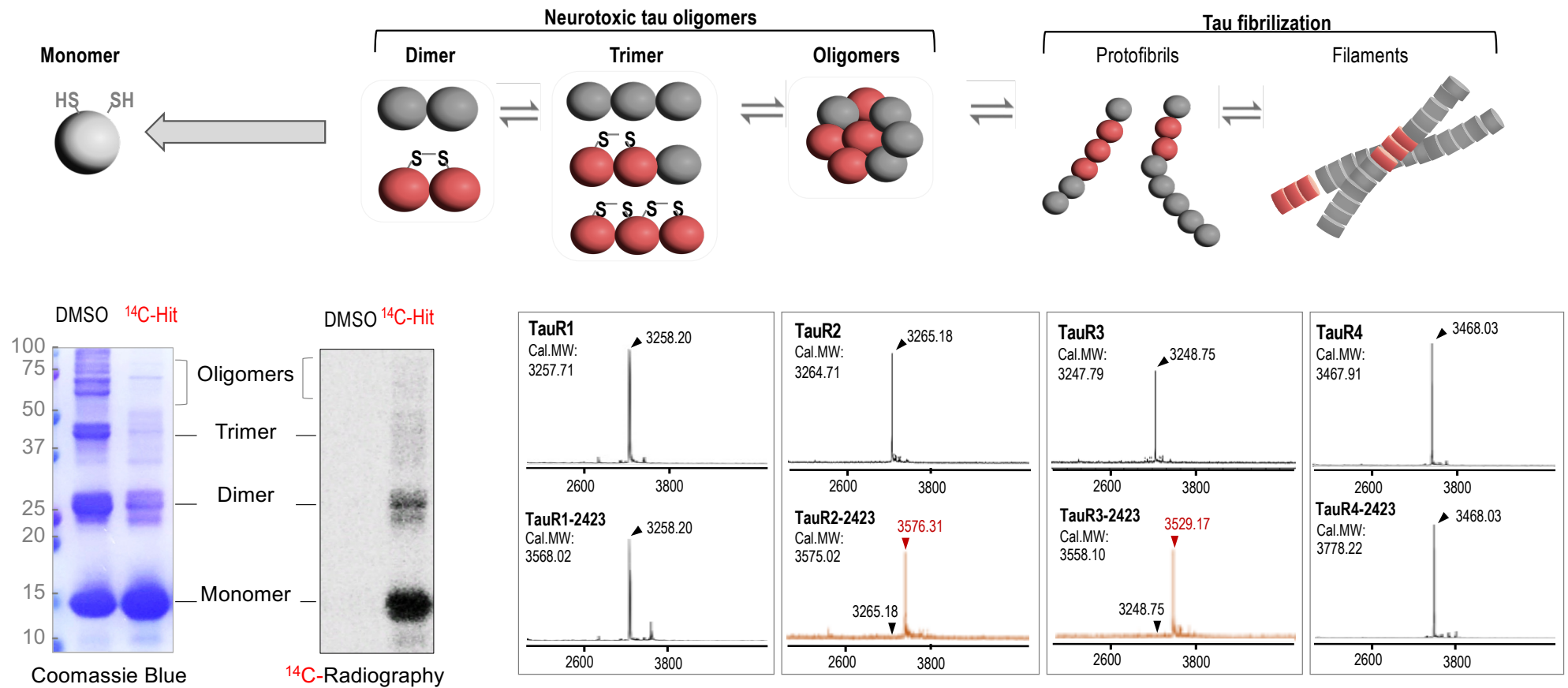
Distance analysis between top tau fibril layers L1-L2

1 μ s MD simulation reveals tau fibril destabilization upon covalent binding to C322

3R/4R tau hit binding disrupts β 4-5 sheet in top layer at 1000ns



Inhibition of Disulfide-linked tau aggregation



In vitro ADME/Tox & PK



Category	Study	DTC2425	DTC2417	DTC2418		
In vitro ADME	CYP450 Remaining Activity (%)	• CYP1A2 (55.6), CYP2C9 (48%), CYP2C19(51.8), CYP2D6(57.5), CYP3A4 (55.4)	• CYP1A2 (92.3), CYP2C9 (100), CYP2C19(98.4), CYP2D6(100), CYP3A4 (96.7)	• CYP1A2 (99.9), CYP2C9 (87), CYP2C19(100), CYP2D6(92.1), CYP3A4 (96.7)		
	Liver Microsomal Stability Remaining Activity (%) (@30 min)	• Human (>100), rat (82.9), Mouse (95.4)	• Human (90), rat (66.1), Mouse (87.3)	• Human (79.8), rat (49.6), Mouse (89.5)		
In vitro Tox	hiPS-CardioMyocyte test	• No toxicity	• No toxicity	• No toxicity		
	hERG (IC50, uM)	• 19.5	• 7.5	• 11.0		
	AMES (TA98, TA100, TA1535, TA1537, Ecoli)	• Negative	• Negative	• Negative		
PK (ICR mice)	IV (2mg/kg)	AUCall (hr*ng/mL)	• 130.1±35.5	• 1701.34	• 4090.37	
		T1/2 (hr)	• 0.2	• 0.58	• 0.51	
		CL(mL/min/kg)	• 131.5±30.9	• 19.5	• 8.33	
		Vss (L/Kg)	• 1.8±0.5	• 0.98(Vz)	• 1.8±0.5	
	PO (10mg/kg)	AUCall (hr*ng/mL)	• 2,214.2±163.3	• 9,868.51	• 23,312.94	
		T1/2 (hr)	• 1.6±0.4	• 0.89	• 1.87	
		Cmax (ng/mL)	• 2,046.6±567.8	• 4,881.41	• 11,145.04	
		Tmax(hr)	• 0.4±0.1	• 0.25	• 0.25	
		CL(mL/min/kg)		• 46.5		
		Vss(L/kg)		• 3.6(Vz)	• 0.283	
		Bioavailability(F, %)	• 113	• 42	• 113.99	
	PK(BBB)	IV (5mg/kg)	0.25 h (ng/mL)	• 83.2	• 22.36	• 138.86
			0.5 h (ng/mL)	• 33.8	• 3.95	• 94.58
PO (10mg/kg)		0.5 h (ng/mL)	• 47.5	• ND	• 73.73	



First line drug candidates targeting 3R4R Tau



DTC0100



Korea: 10-1910153
U.S. : 15/697,728

DA-7503



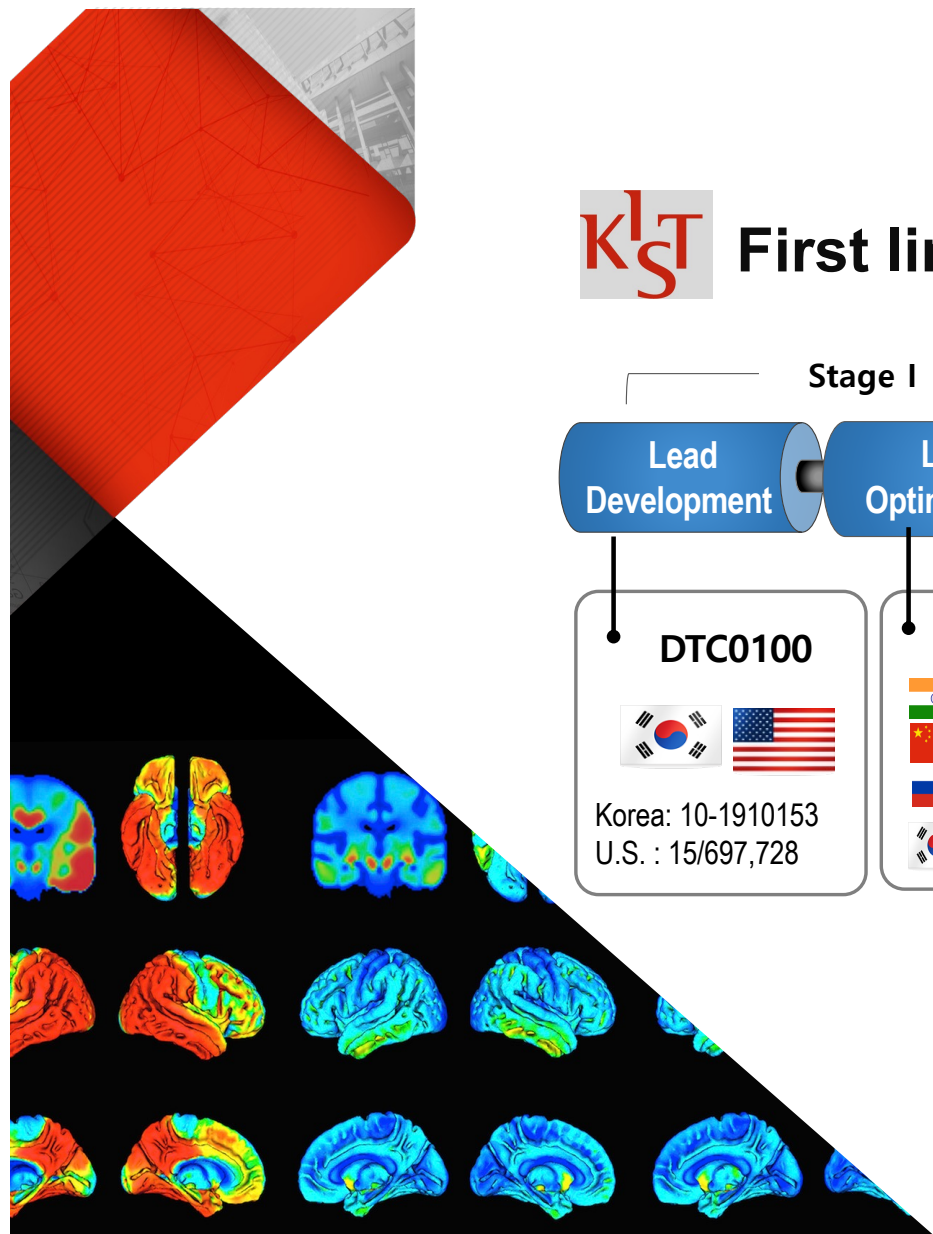
Patent 13



DONG-A PHARM.

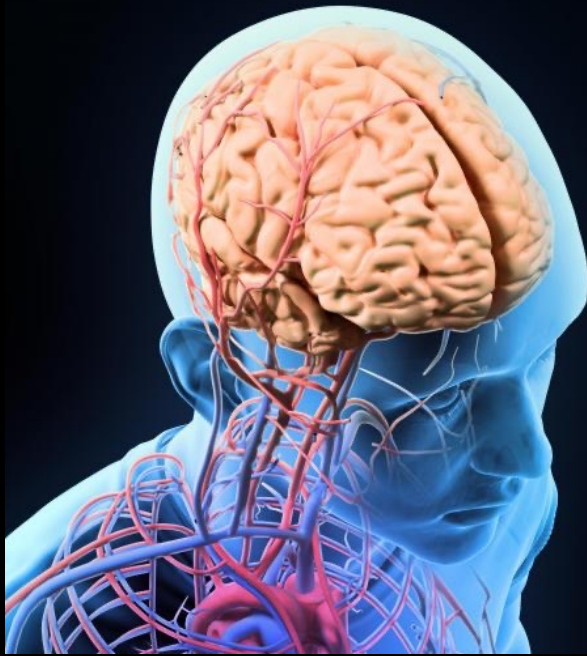
DA-7503 Licensing-Out

**2024 May,
DA-7503
Clinical Trial I**



Brain has been recognized as a “Bacterial Free” area

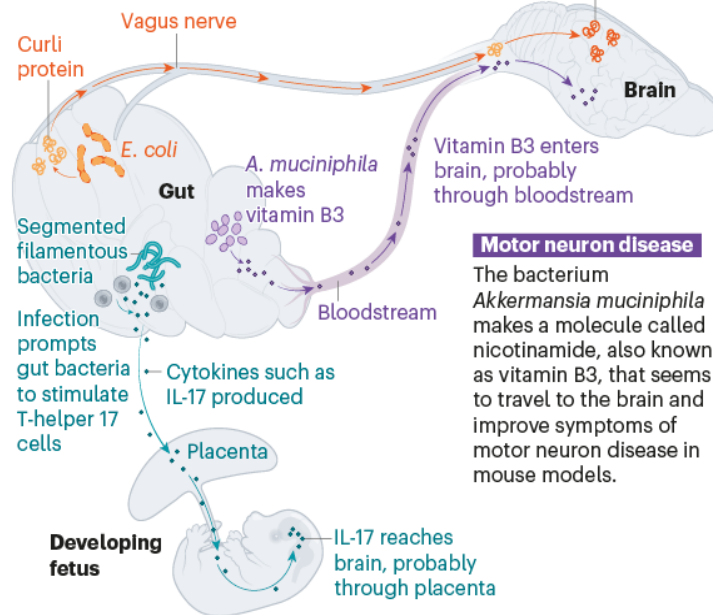
Blood-Brain Barrier



Gut-Brain axis

Parkinson's disease

A strain of *Escherichia coli* in the gut makes a protein called curli, which can prompt other proteins, including one called α -synuclein, to misfold. Some researchers suspect that these misfolded proteins transmit the error up the vagus nerve to the brain, where misfolded α -synuclein is linked to disease symptoms.



Motor neuron disease

The bacterium *Akkermansia muciniphila* makes a molecule called nicotinamide, also known as vitamin B3, that seems to travel to the brain and improve symptoms of motor neuron disease in mouse models.

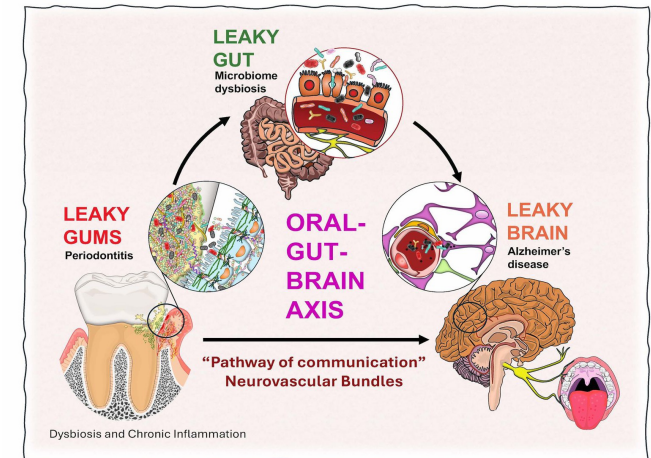
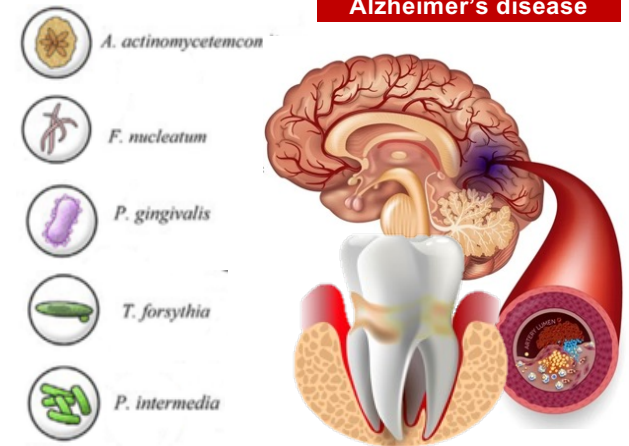
Autism spectrum disorder

Studies in mice suggest an infection in pregnancy can set off a cascade of activity. In the mother's gut, segmented filamentous bacteria stimulate T-helper 17 cells — part of the immune system. They produce immune molecules that travel to the fetus's brain and provoke autism-like behaviour.

©nature

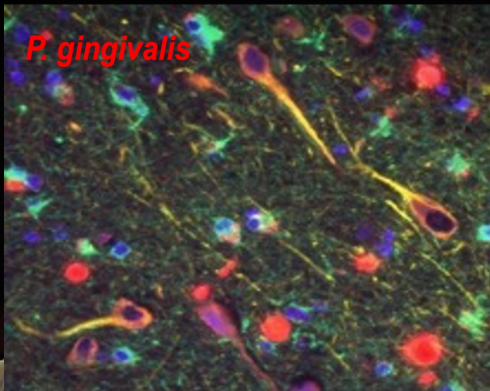
Oral-Brain axis

Alzheimer's disease

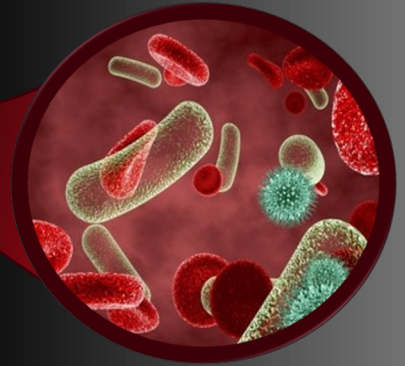
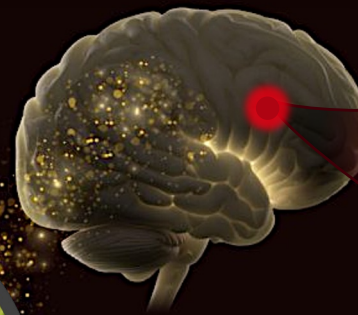


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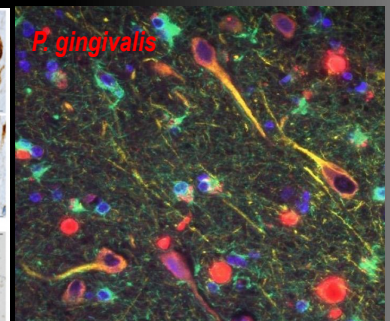
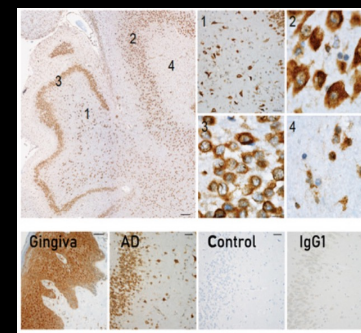
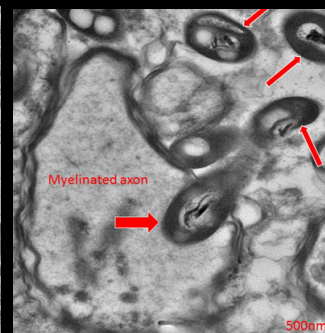
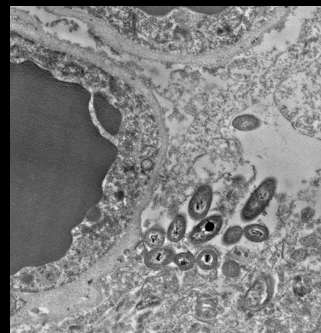
P. Gingivalis detected in the AD brain



Classically,
Bacteria-secreted molecules
affect brain



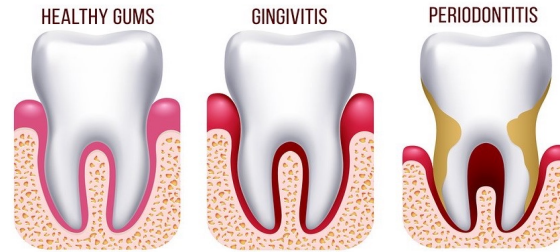
Recently,
Bacteria found in the brain



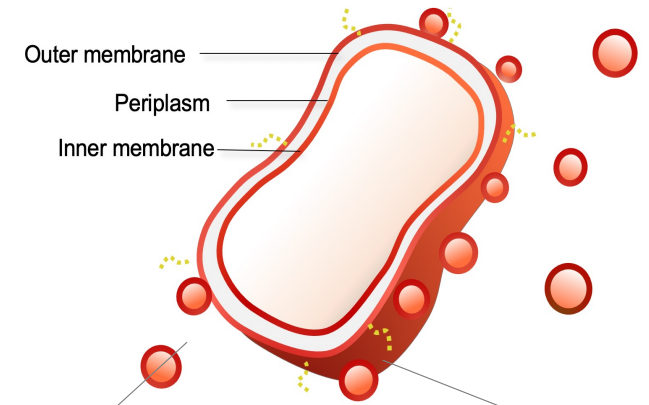
34 cases of normal & schizophrenia (Neuroscience 2018). *P. gingivalis* infection in AD brain (Sci. Adv., 2019)

Why Gingivitis?

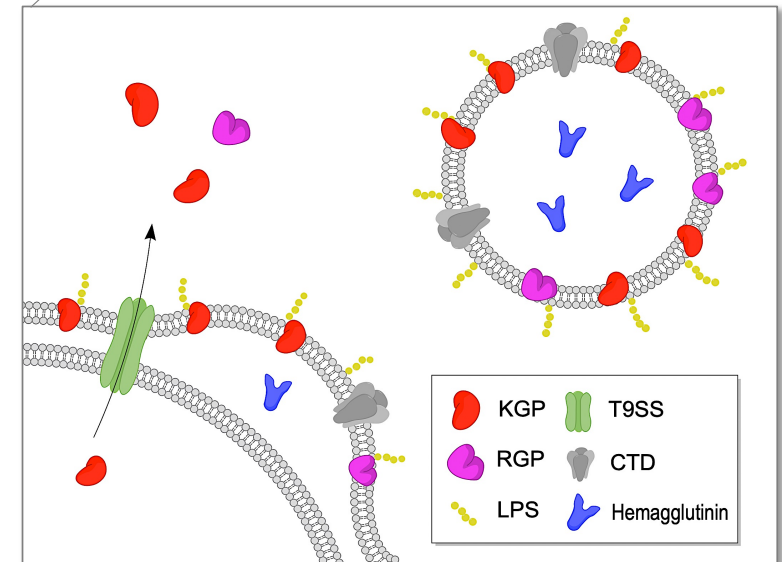
It secretes strong Cysteine proteases



	아그레가티박터 악티노마이세툼코미탄스 <i>Aggregatibacter actinomycetemcomitans</i>	Aa	침습성 치주염을 유발하며 조직의 파괴를 촉진	초고위험군
	포피노모나스 진지발리스 <i>Porphyromonas gingivalis</i>	Pg	잇몸의 염증 및 치조골 파괴	고위험군
	테너엘라 포시티아 <i>Tannerella forsythia</i>	Tf	난치성 치주염을 유발	고위험군
	트레포네마 덴티콜라 <i>Treponema denticola</i>	Td	잇몸의 염증 및 치조골 파괴	고위험군
	프레보텔라 인터미디아 <i>Prevotella intermedia</i>	Pi	호르몬 관련성 치주염을 유발하여 복합 감염 치주염 촉진	중위험군
	푸소박테리움 뉴클레아툼 <i>Fusobacterium nucleatum</i>	Fn	급성 괴사성, 궤양성 치은염을 유발	중위험군
	파비모나스 마이크라 <i>Parvimonas micra</i>	Pm	치주염의 진행과 활동을 활발하게 하여 치주조직의 파괴를 유발	중위험군
	캄필로박터 렉투스 <i>Camphylobacter rectus</i>	Cr	치근막질환을 유발하여 치주질환으로 발전	중위험군
	락토바실러스 아시도필루스 <i>Lactobacillus acidophilus</i>	La	젖산을 분비하여 치아우식(충치)으로 발전	충치관련군
	스트렙토코커스 뮤탄스 <i>Streptococcus mutans</i>	Sm	플라그(치태)를 형성하여 치아우식(충치) 촉진	충치관련군
	스트렙토코커스 소브리누스 <i>Streptococcus sobrinus</i>	Ss	법랑질을 탈회하여 치아우식(충치) 유발	충치관련군



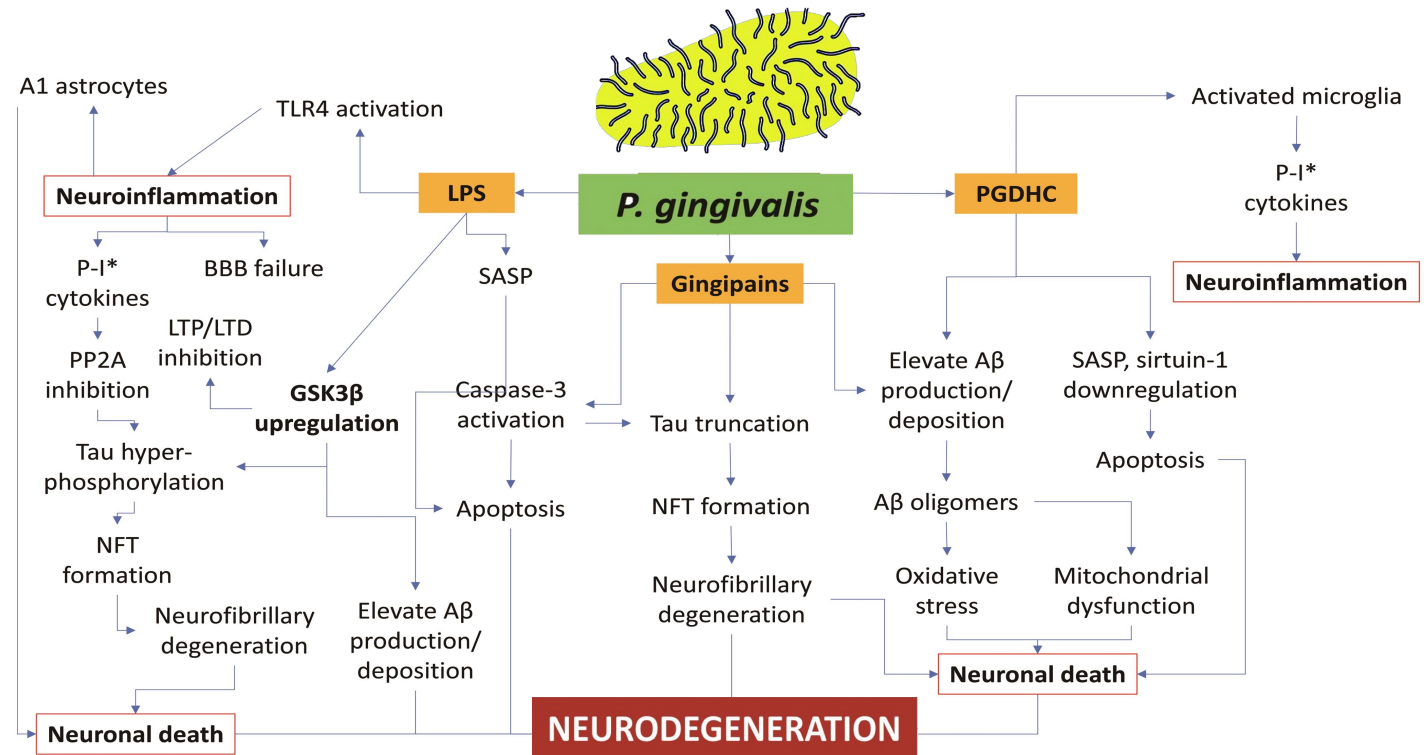
Gingipains: Cysteine protease



Gingivalis in the brain

-neuro-inflammation

-neuro-degeneration

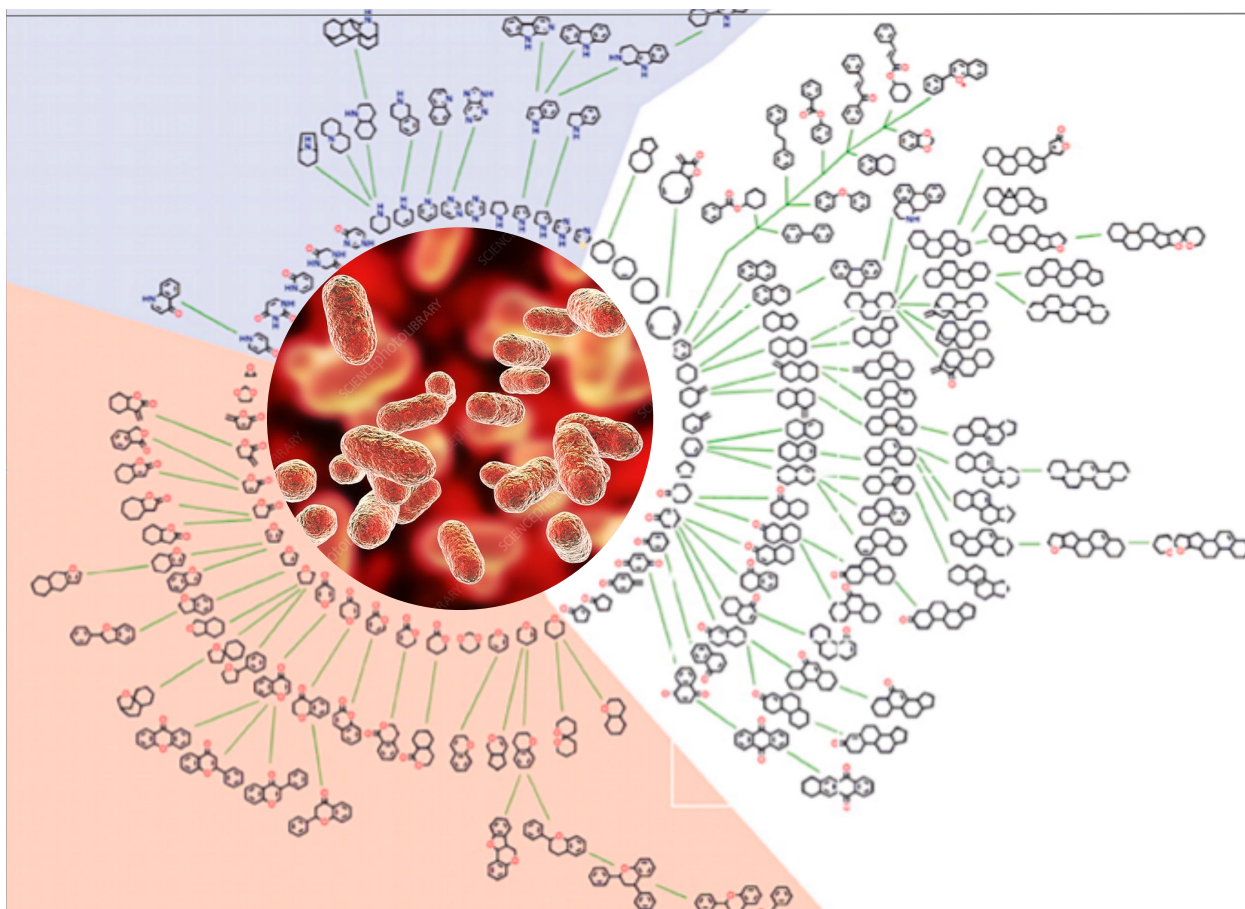


Journal of Exploratory Research in Pharmacology 2022;7(1):45-53

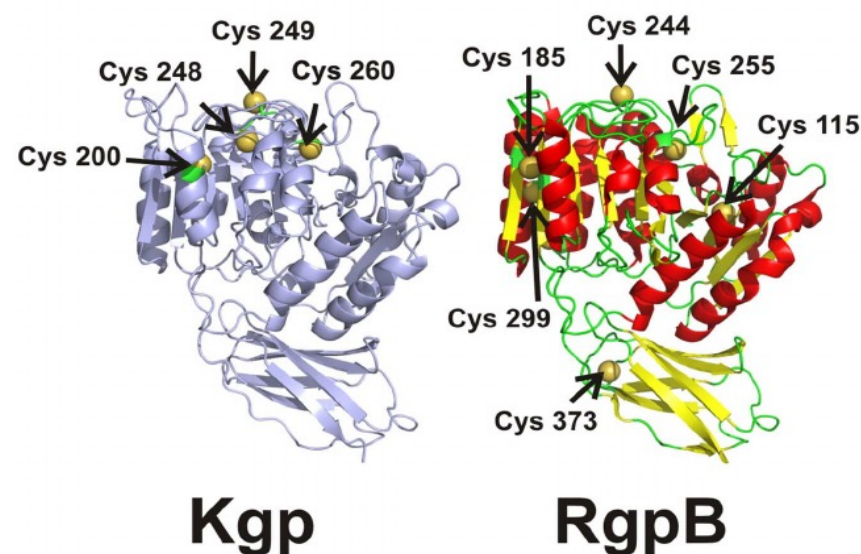




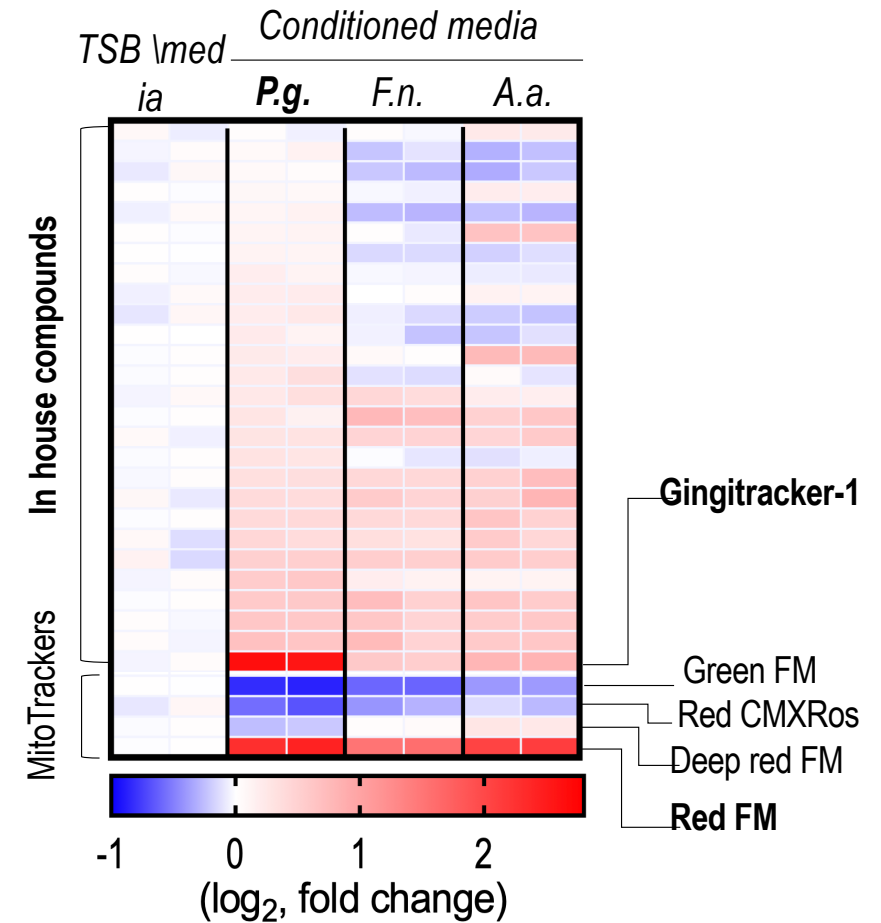
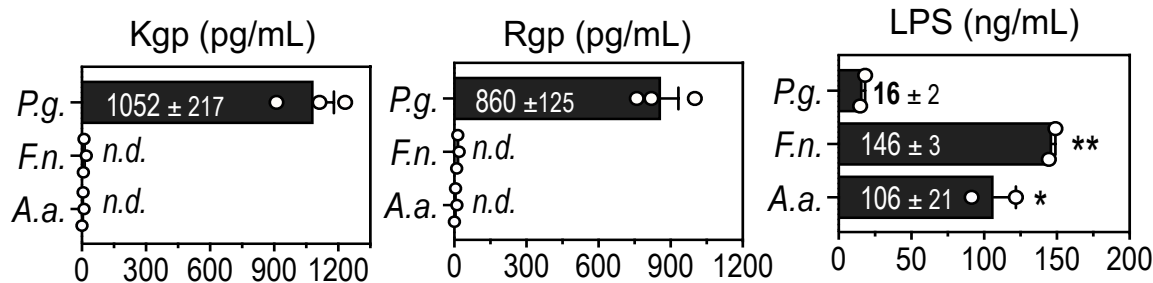
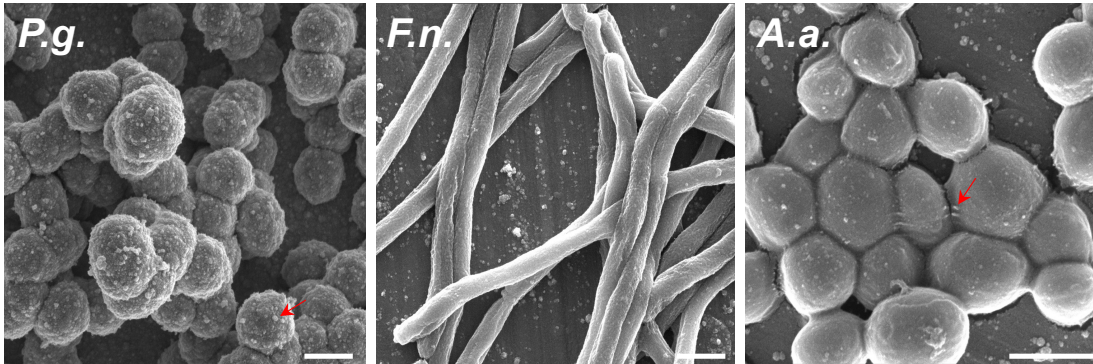
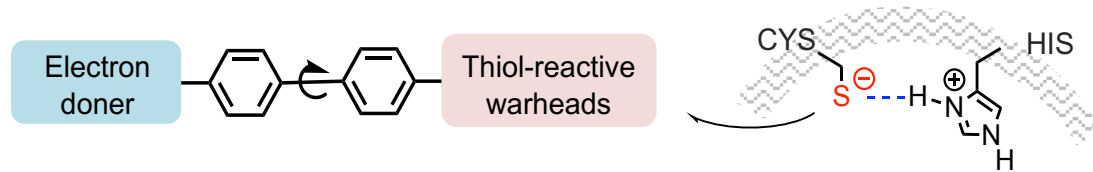
2600' Cysteine Targeted Druggable library compounds



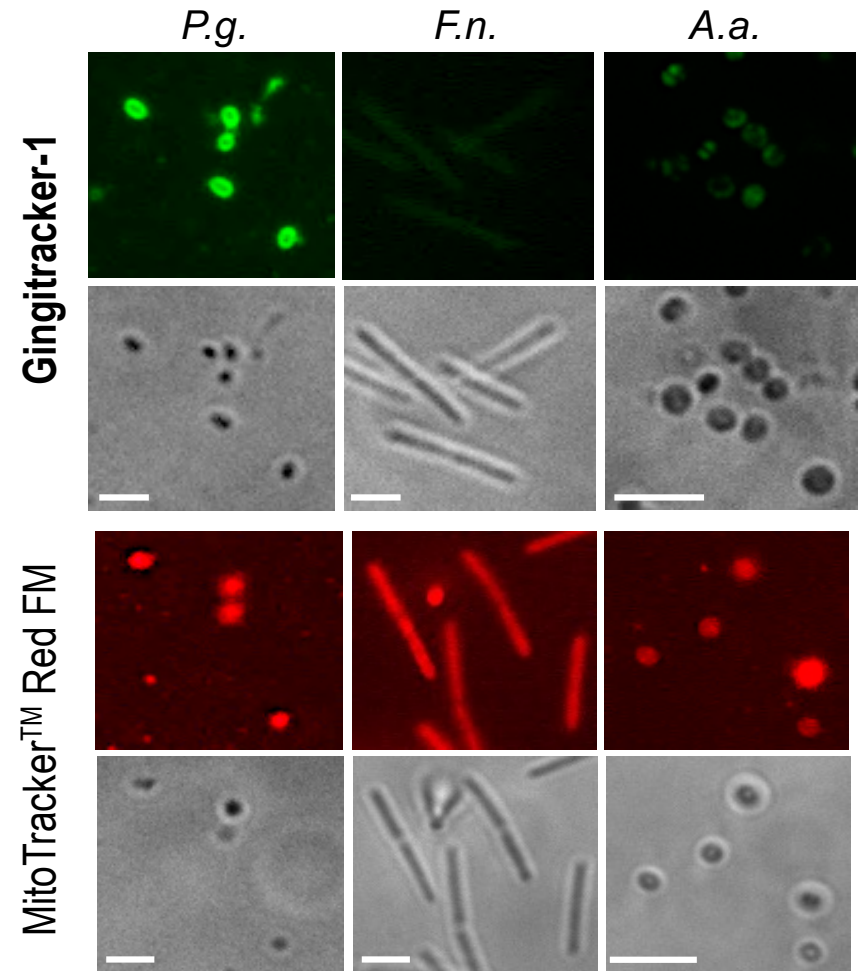
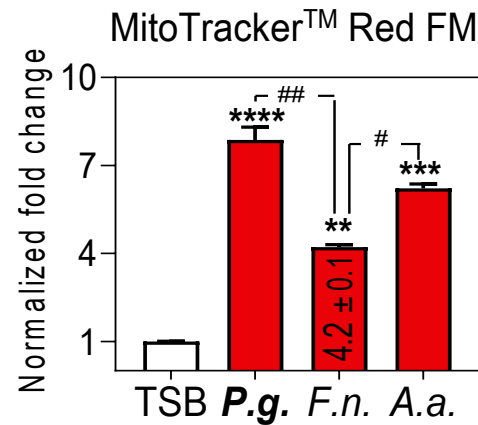
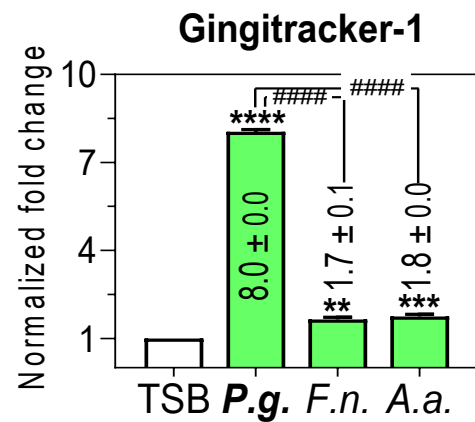
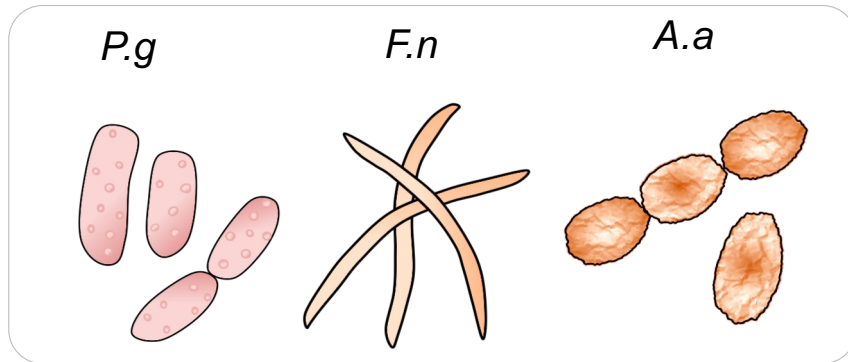
Gingipains *Cysteine Protease*



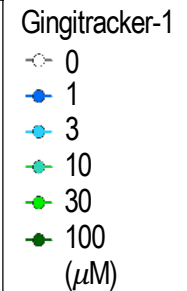
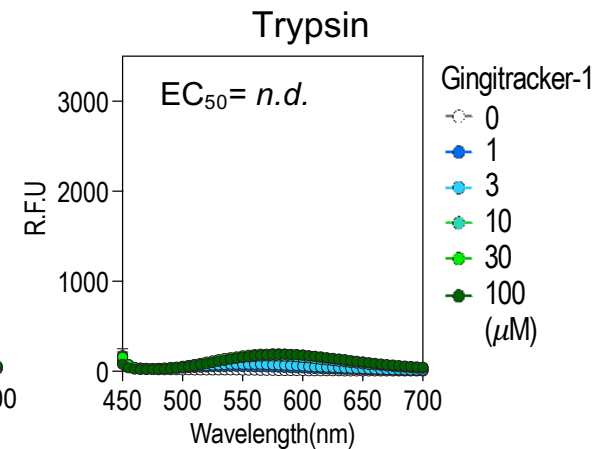
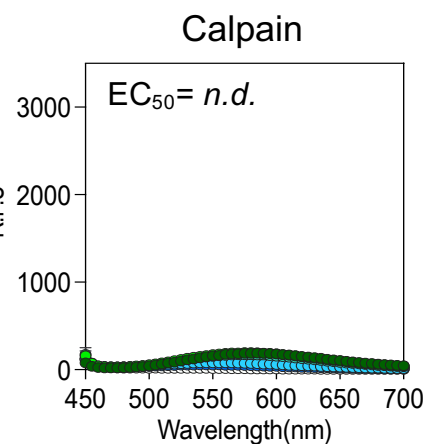
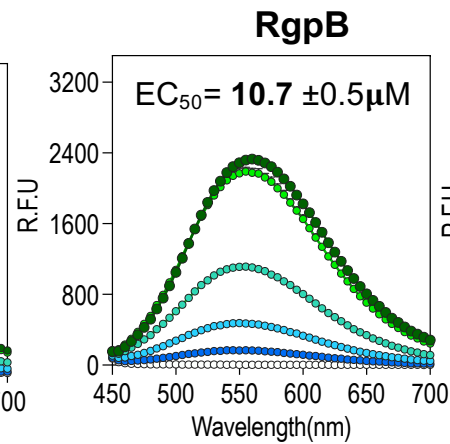
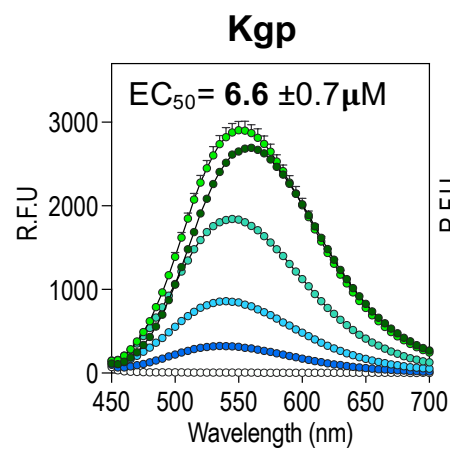
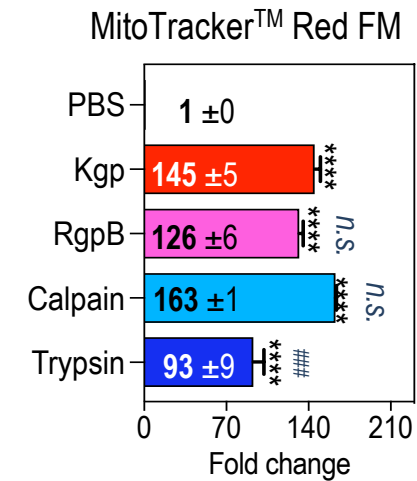
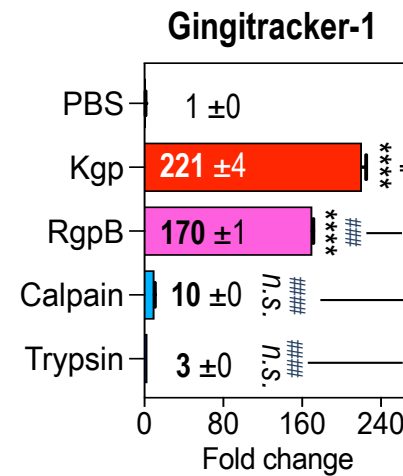
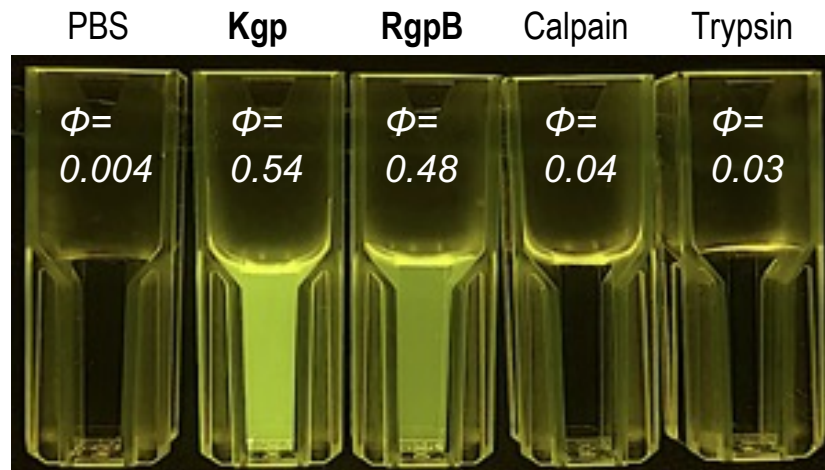
Screening of *P. gingivalis*-selective probes



Gingitracker-1: Selectivity to *P. gingivalis*



Gingitracker-1: Selectivity to gingipains



Gingitracker-1: inhibits gingipain activities

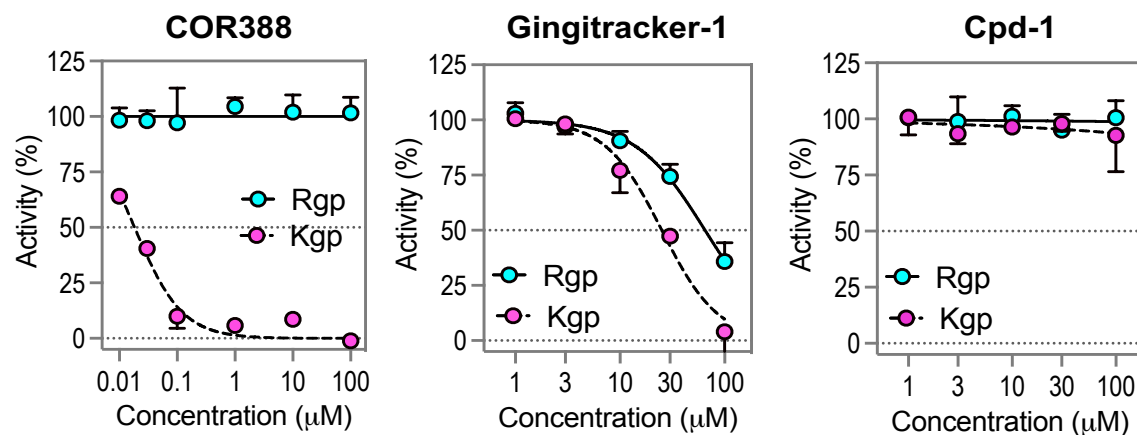
치주염에서 잇몸 출혈



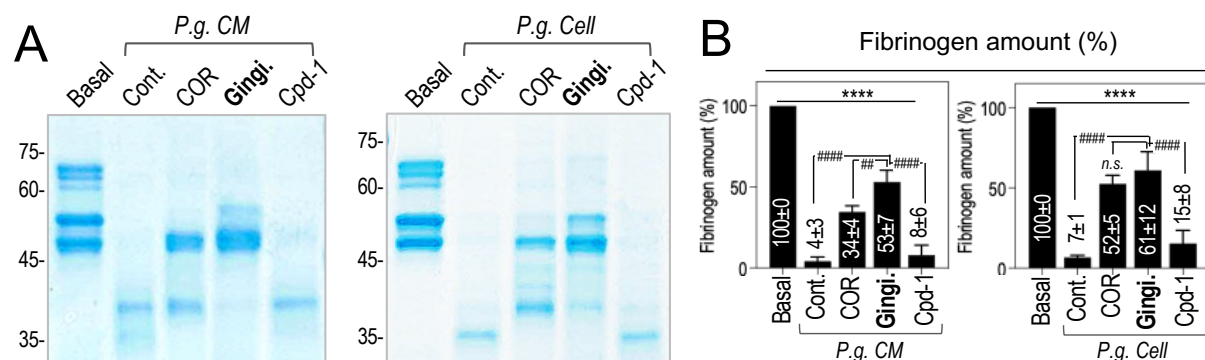
피브리노겐에 의한 혈액응고



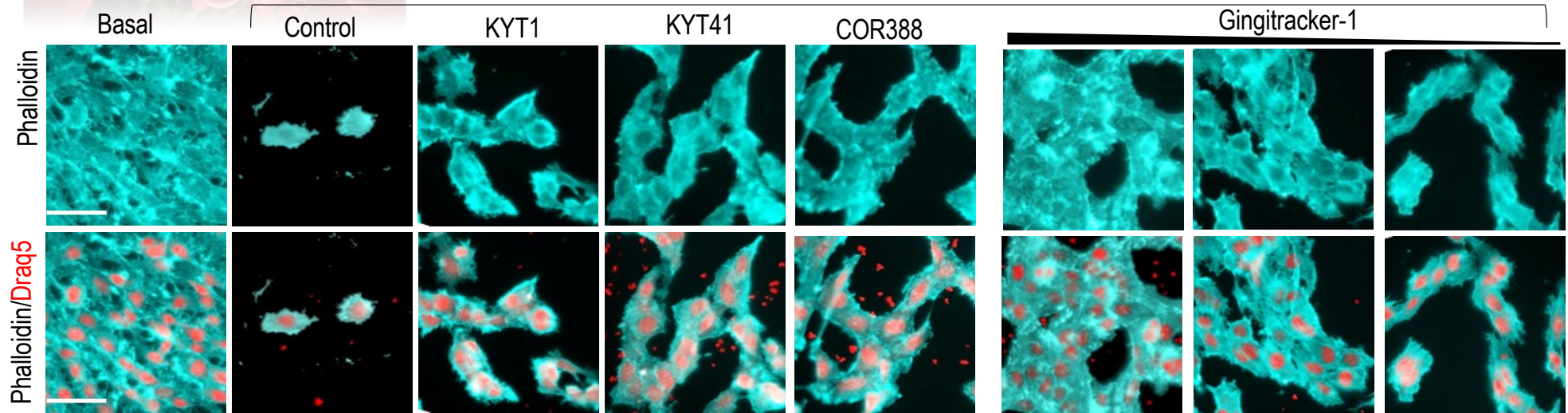
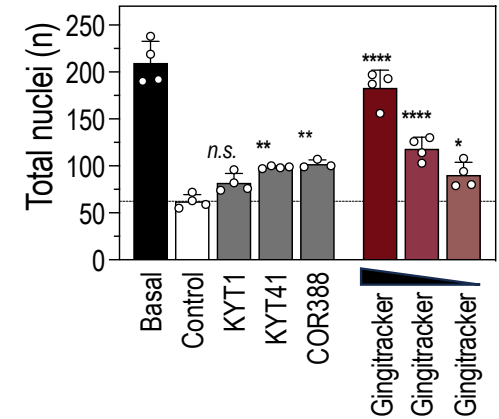
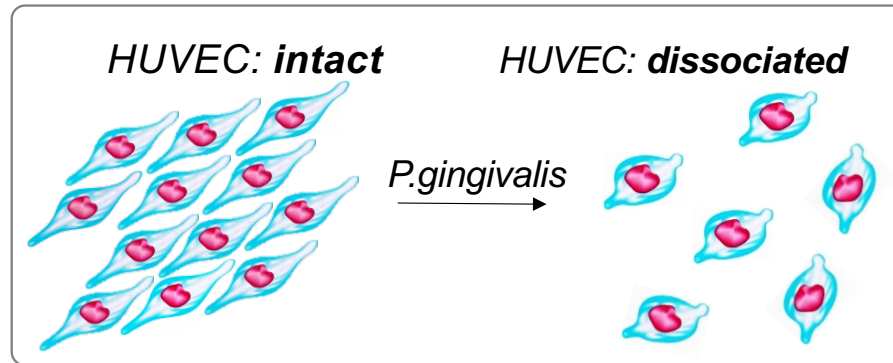
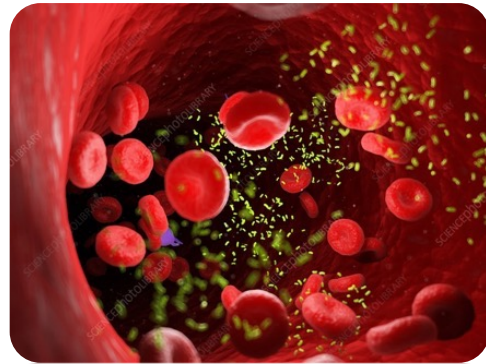
Inhibition of Kgp / Rgp enzyme activity



Inhibition of Fibrinogen Cleavage by *P. Gingivalis*

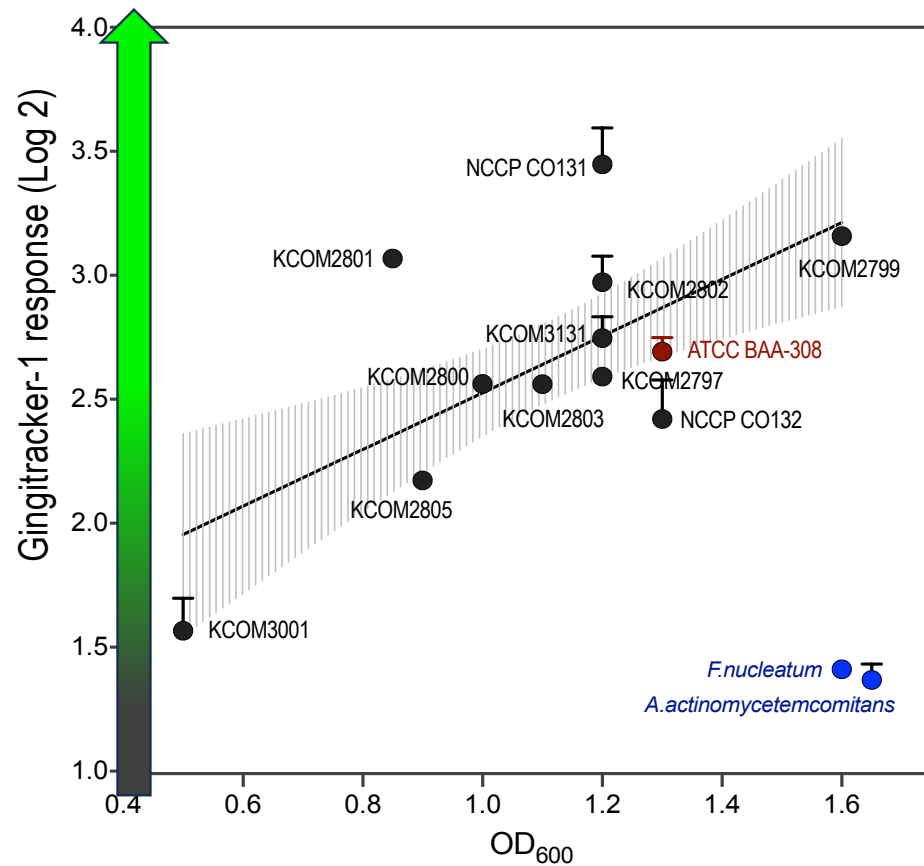


Gingitracker-1: Inhibits HUVEC dissociation by P.G.



Human periodontitis : Detection of Gingipains

Korean 11 *P. gingivalis* strains



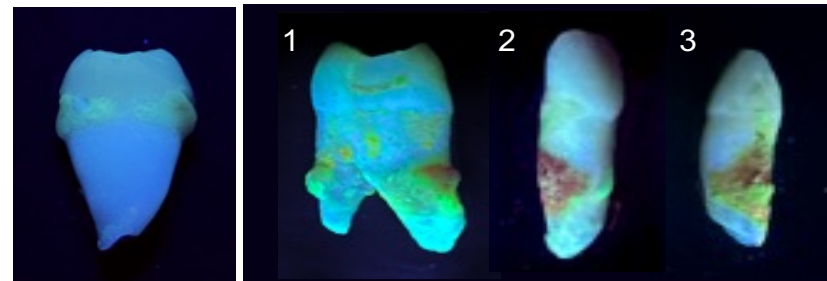
Normal tooth



Human periodontitis teeth



Gingitracker-1



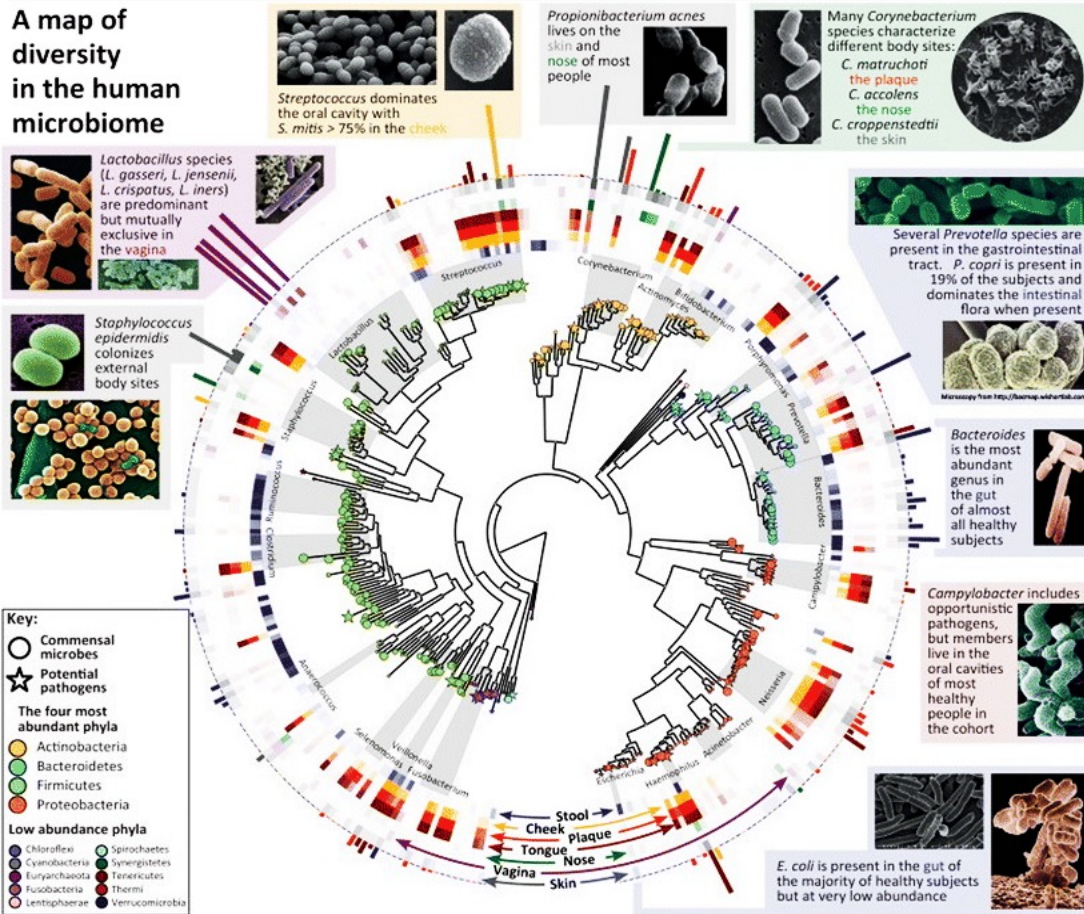
	Kgp (pg/mL)	Rgp (pg/mL)	Gingitracker-1 (R.F.U)
Plaque 1	669	403	473
Plaque 2	168	355	265
Plaque 3	208	528	425

Tau-BiFC cellular screening with 4,228 gut microbial strains



4,228 gut microbial strains

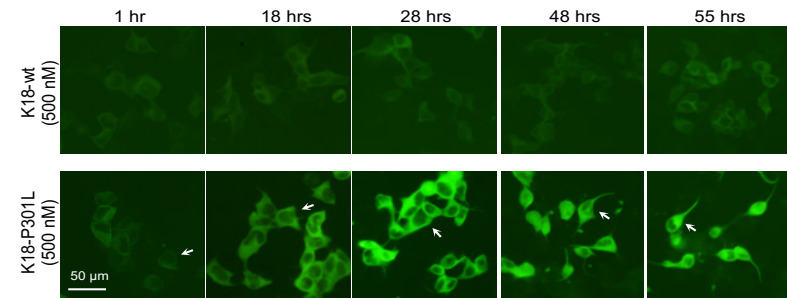
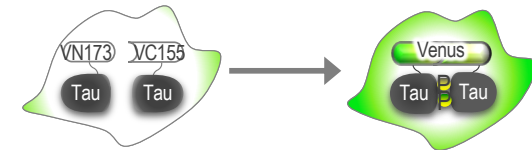
A map of diversity in the human microbiome



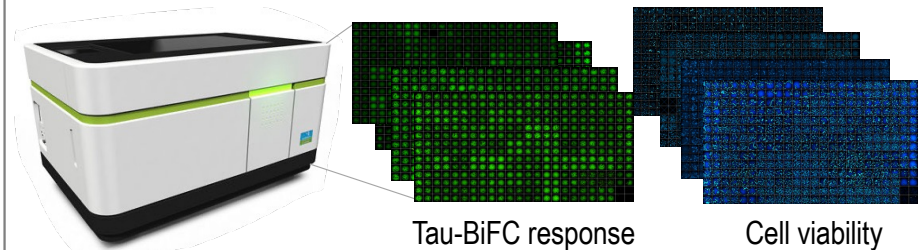
Tau-BiFC HTS screening

Tau-BiFC "OFF"

Tau-BiFC "ON"

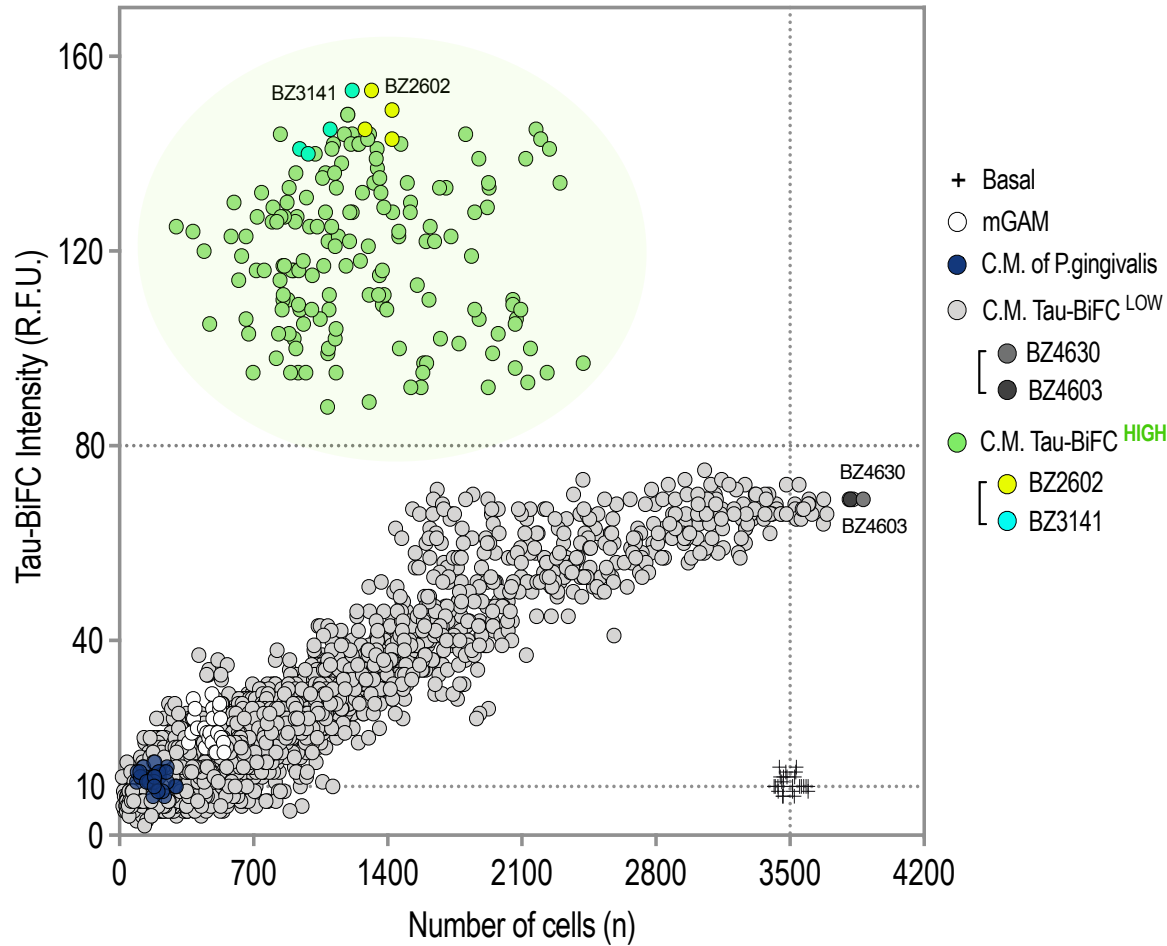


초고속 자동화 이미징 및 분석 (Operetta® CLS)

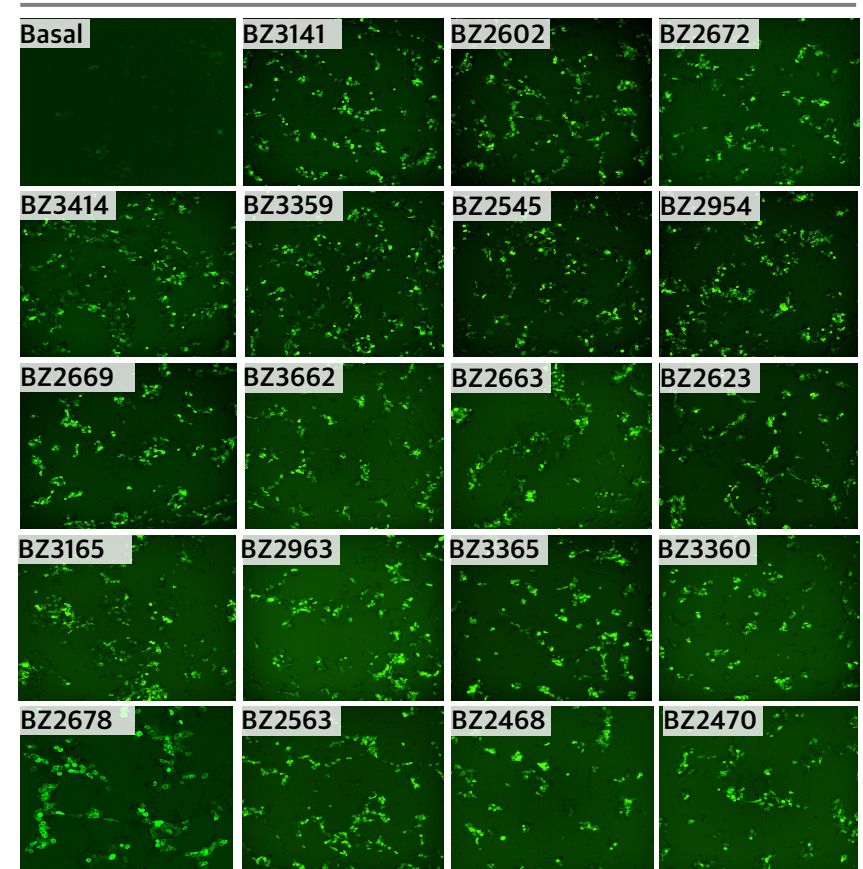


Tau-BiFC cellular screening with 4,228 gut microbial strains

Tau-BiFC screening of **4,228** microbiota C.M.



Acquisition of culture supernatants from 19 harmful bacterial strains



THANK YOU

