

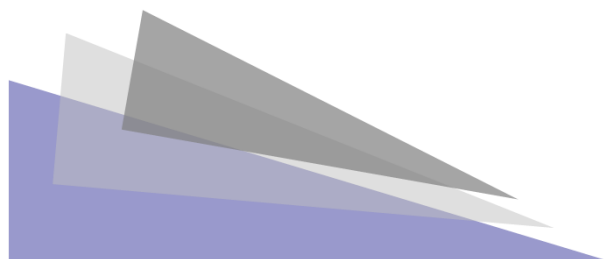
「ディープフェノタイピング法に基づく化学物質の生物作用分析システムの開発」

楠原 洋之

東京大学大学院薬学系研究科

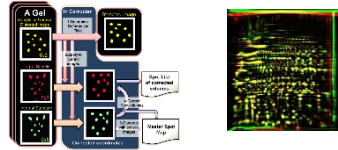
分子薬物動態学教室

21/Aug/2020

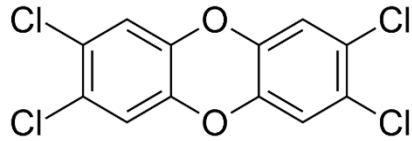
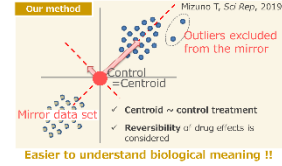


Summary

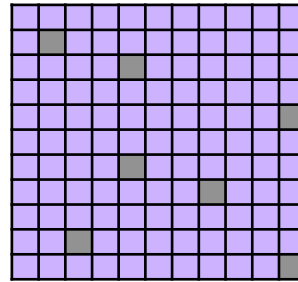
バイアスのない変換



教師なし解析

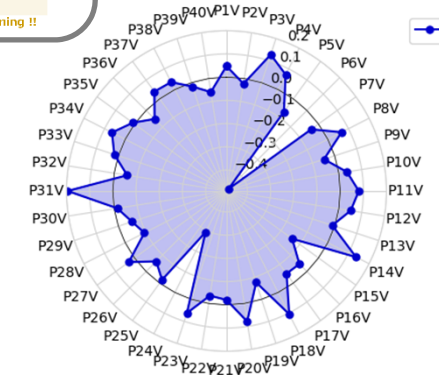


生物学的情報



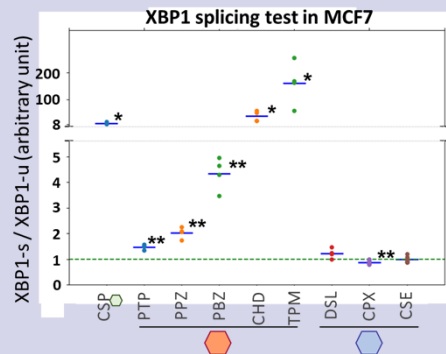
数値的情報

= プロファイルデータ

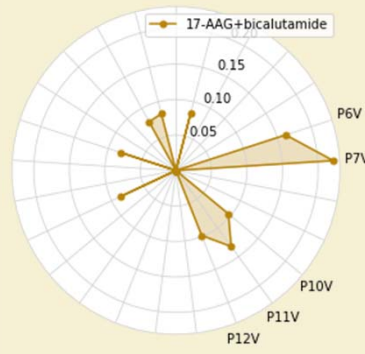


化学物質の作用分離

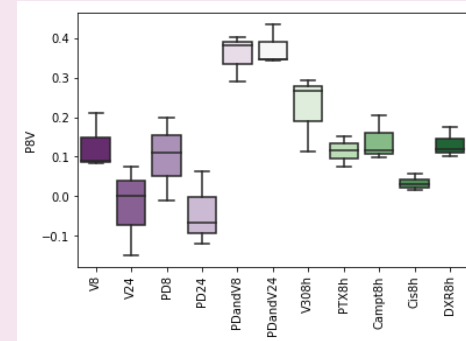
① (未知の作用も含めた) 恣意性のない作用評価



② 複合的な作用の評価



③ 相乗効果の評価



1. 化学物質が持つ作用のバイアスのない変換

- ✓ ハンドリングが容易な二次元電気泳動装置の開発

2. 教師無し解析による化学物質の作用分離

- ✓ 化学物質の作用分離解析手法の開発

3. 何ができるか？

- ✓ 潜在的な毒性の検出
- ✓ 複合的な作用の分離

4. まとめ



Topic

1. 化学物質が持つ作用のバイアスのない変換

- ✓ ハンドリングが容易な二次元電気泳動装置の開発

2. 教師無し解析による化学物質の作用分離

- ✓ 化学物質の作用分離解析手法の開発

3. 何ができるか？

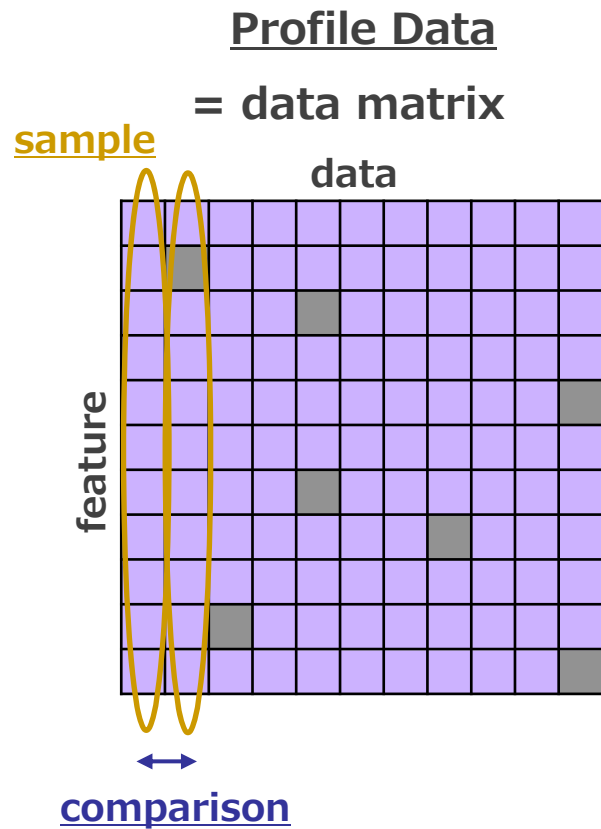
- ✓ 潜在的な毒性の検出
- ✓ 複合的な作用の分離

4. まとめ



Profile data “analysis” is

Analysis of Profile Data Relationship

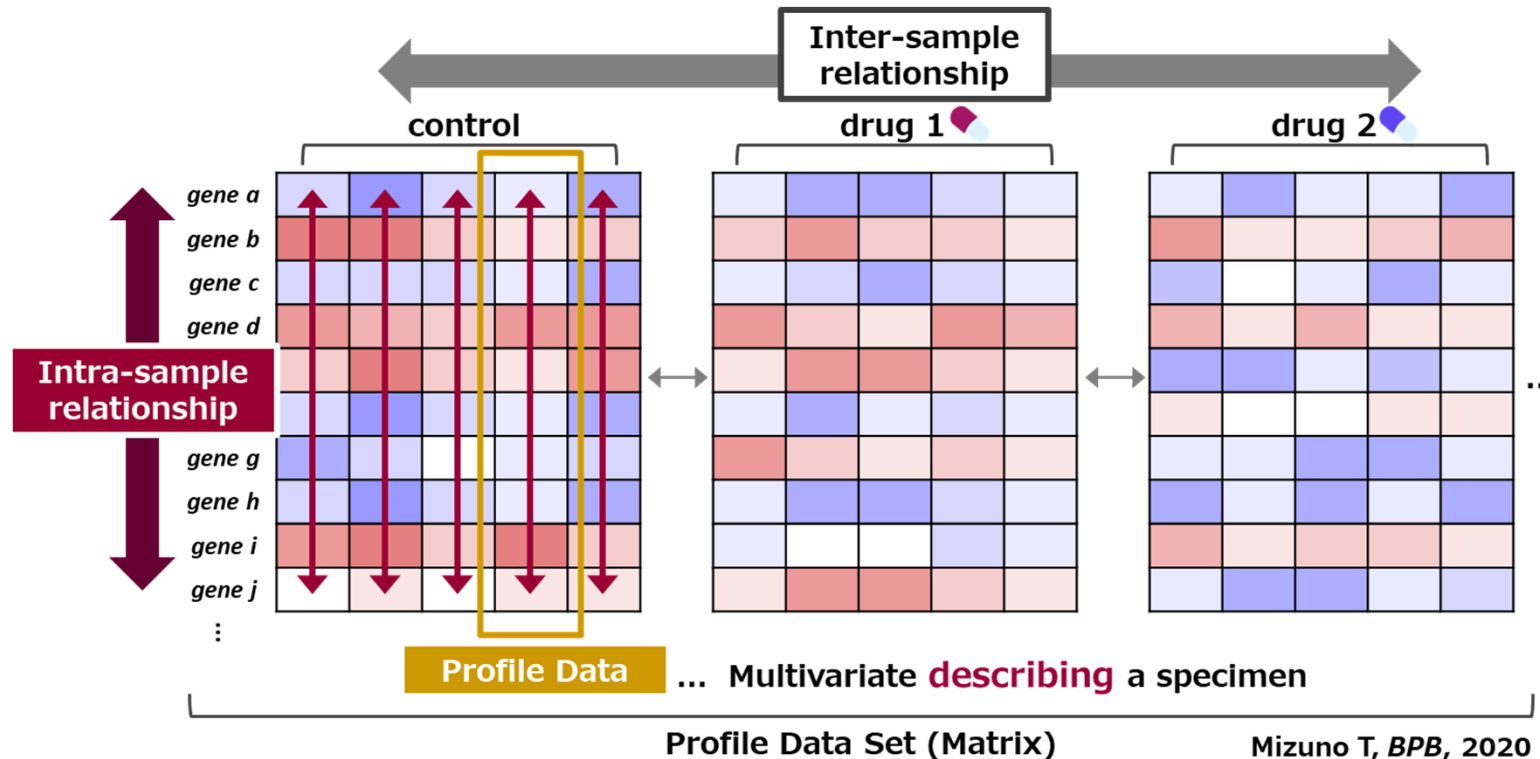


Utilize Data Structure

borrow information of the others

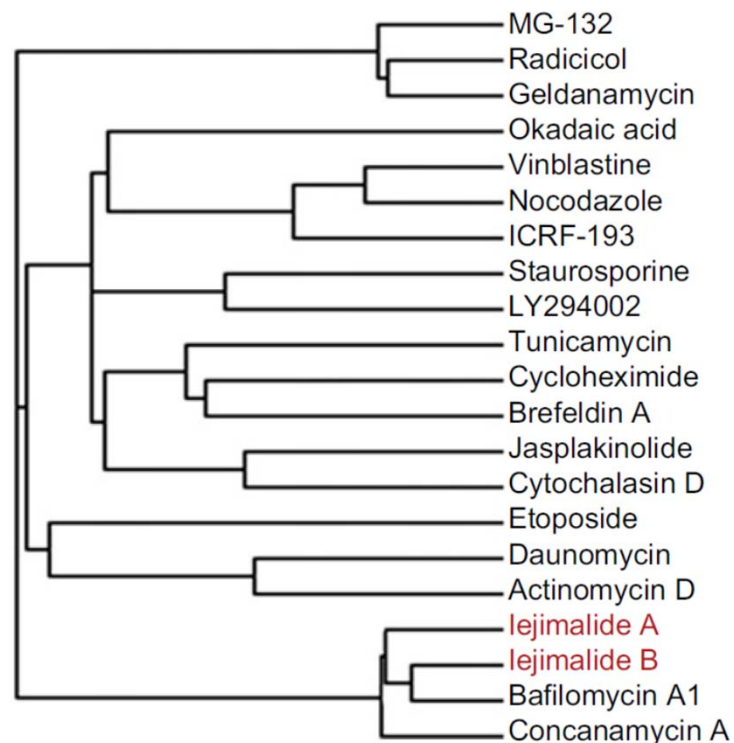
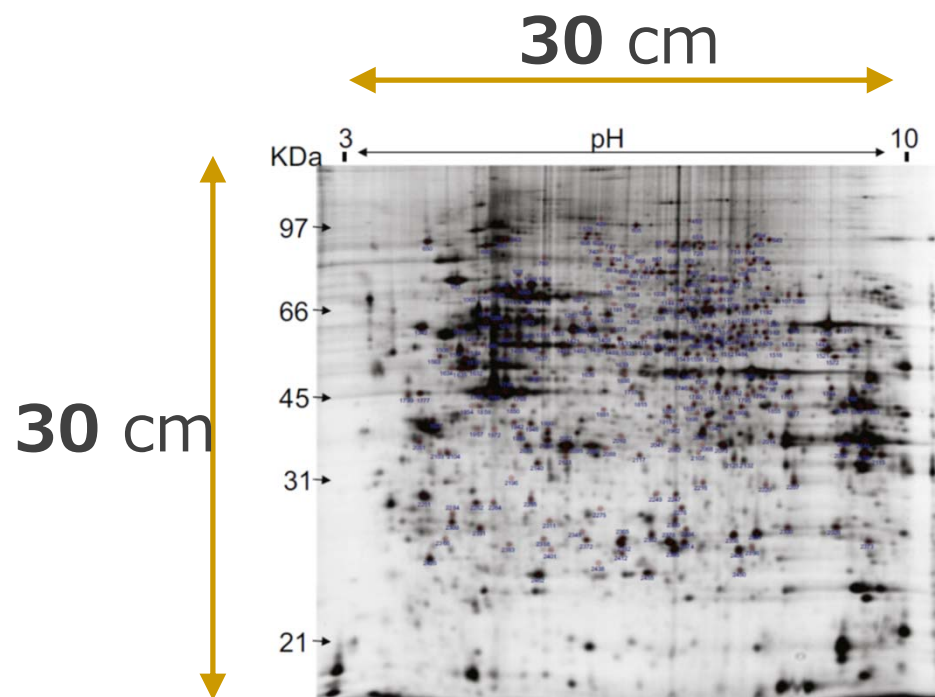
- 1) High Detection Sensitivity
- 2) Robust

オミクス解析による薬剤応答プロファイルデータの取得



二次元電気泳動によるプロテオーム

- ✓ 網羅的で変数選択にバイアスがない
- ✓ 低分子化合物の作用を反映



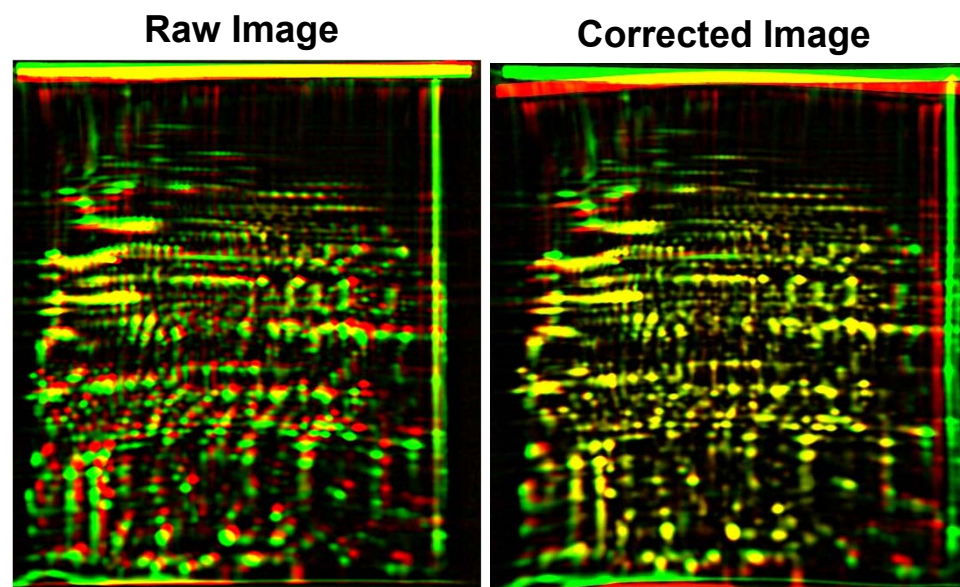
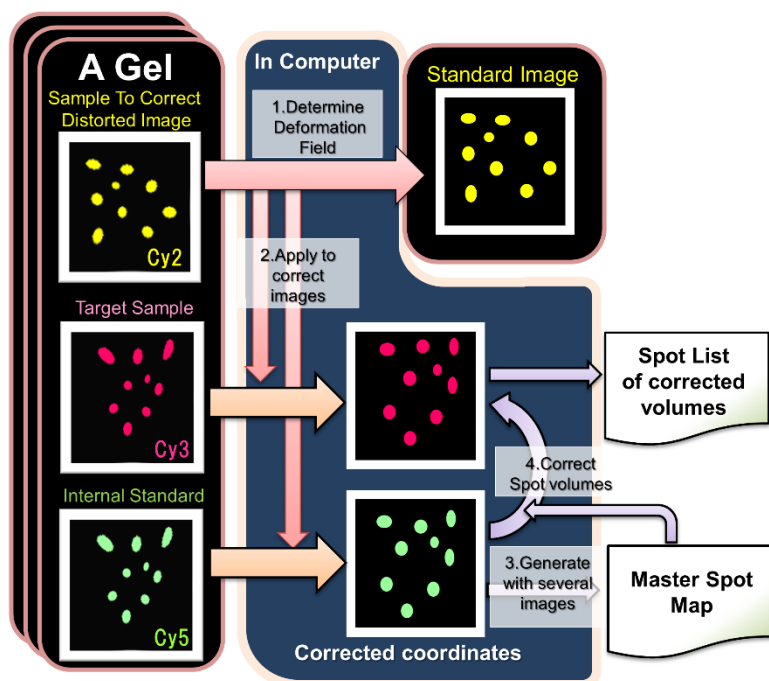
Muroi M, *Chem Biol*, 2010

However,

一般に二次元電気泳動は**ハンドリングに難**がある。

ハンドリングの良い二次元電気泳動法

- ✓ ミニゲルサイズ (10 x 10 cm) でハンドリングの容易な実験系
- ✓ 独自画像解析手法による再現性の高いスポットの検出

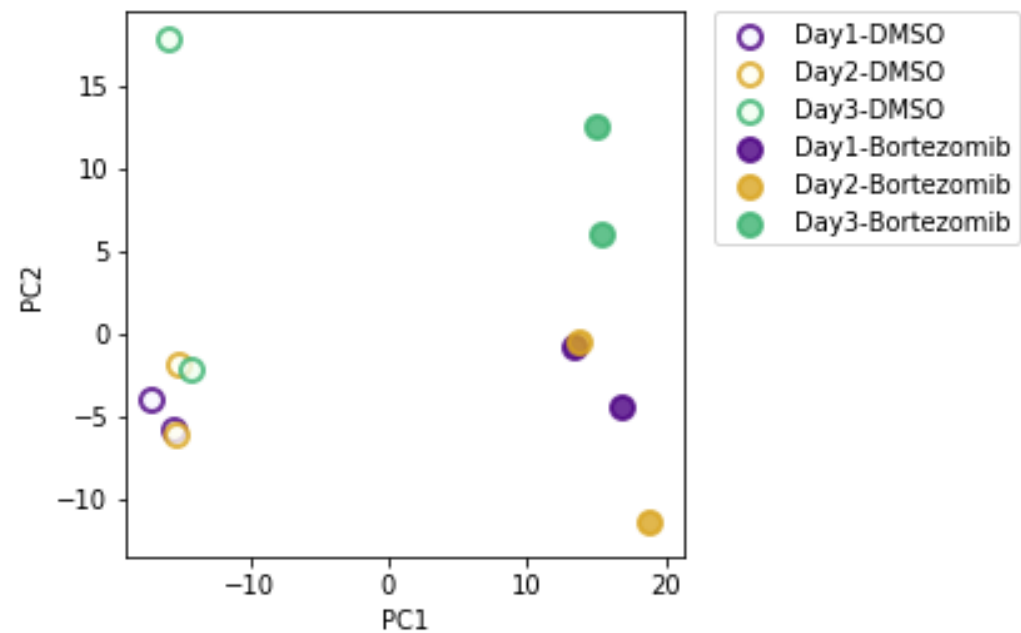
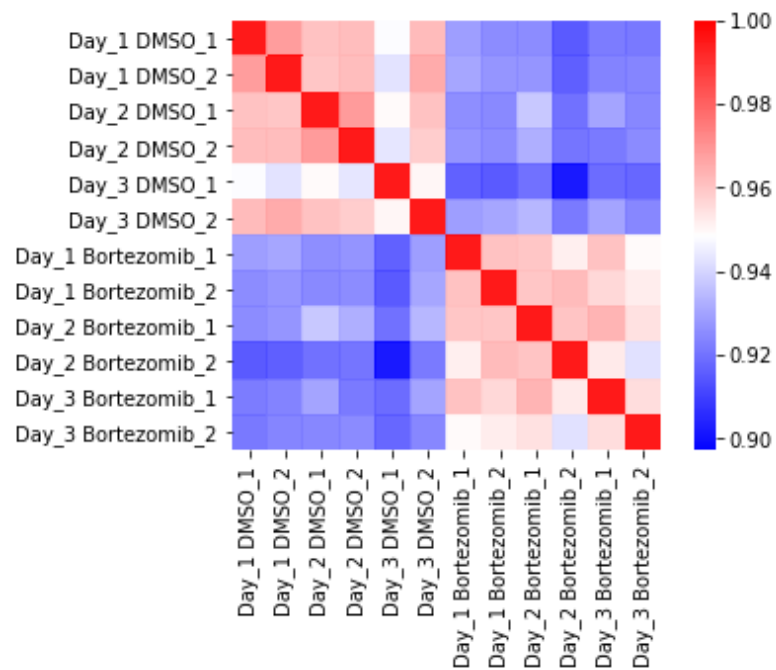


Kinoshita S, *Biol Pharm Bull*, 2019

(プロメディコ社との共同研究)

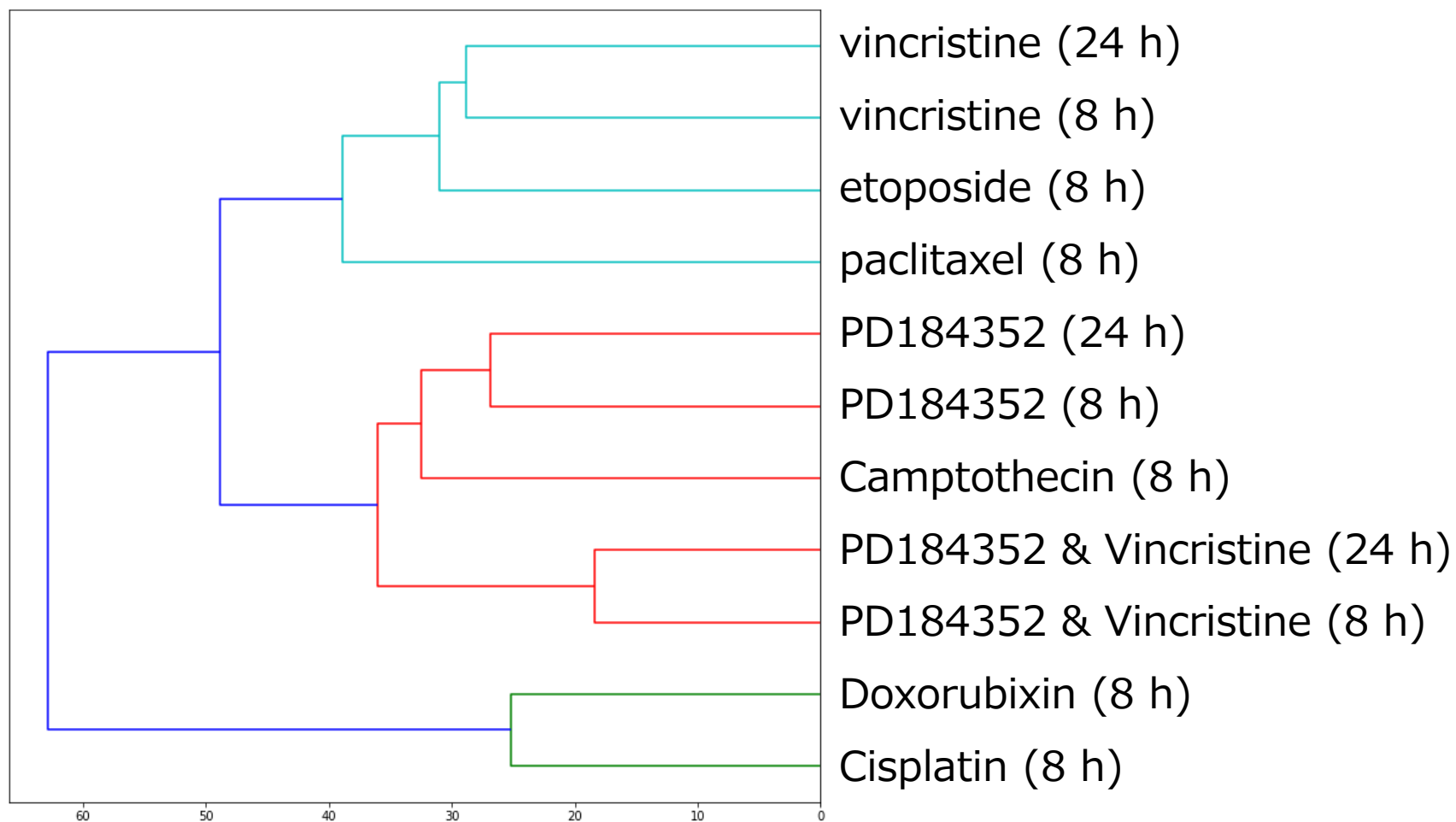
ハンドリングの良い独自二次元電気泳動法

- ✓ 日間差の少ないプロファイルデータ取得に成功
- ✓ 薬物の作用を反映



ハンドリングの良い独自二次元電気泳動法

- ✓ 抗がん剤処理を施した試料のデータを取得
- ✓ **薬物の作用を反映** (解析中)



Topic

1. 化学物質が持つ作用のバイアスのない変換

- ✓ ハンドリングが容易な二次元電気泳動装置の開発

2. 教師無し解析による化学物質の作用分離

- ✓ 化学物質の作用分離解析手法の開発

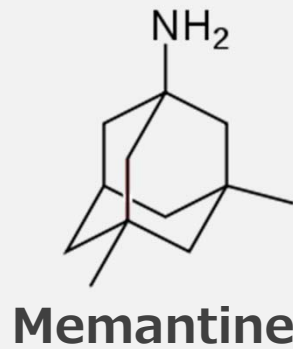
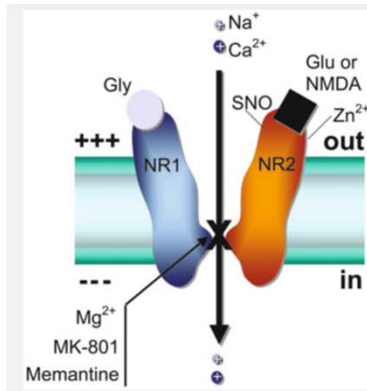
3. 何ができるか？

- ✓ 潜在的な作用の検出
- ✓ 複合的な作用の分離

4. まとめ



Unrecognized aspects of chemicals

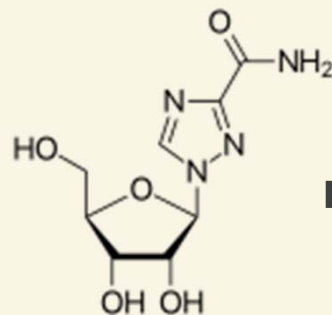


✓ Developed for **anti-flu**

➔ Also used for **Dementia**

Newly targeting
NMDA receptor

Lipton SA, *NeuroRX*, 2004



ribavirin

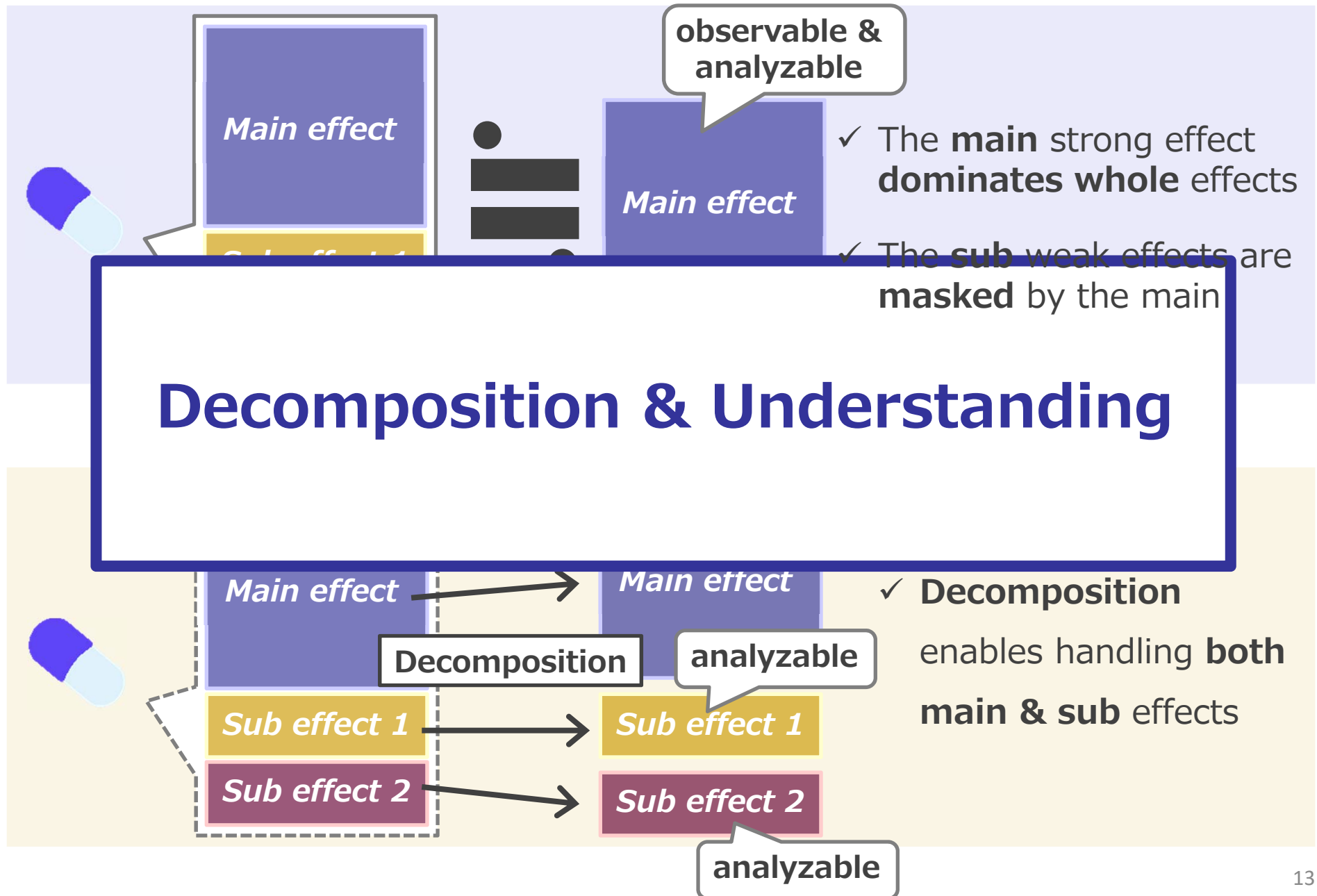
✓ Developed for **anti-viral medication such as HCV**

➔ Also effective for **Malignant Prostate Cancer**

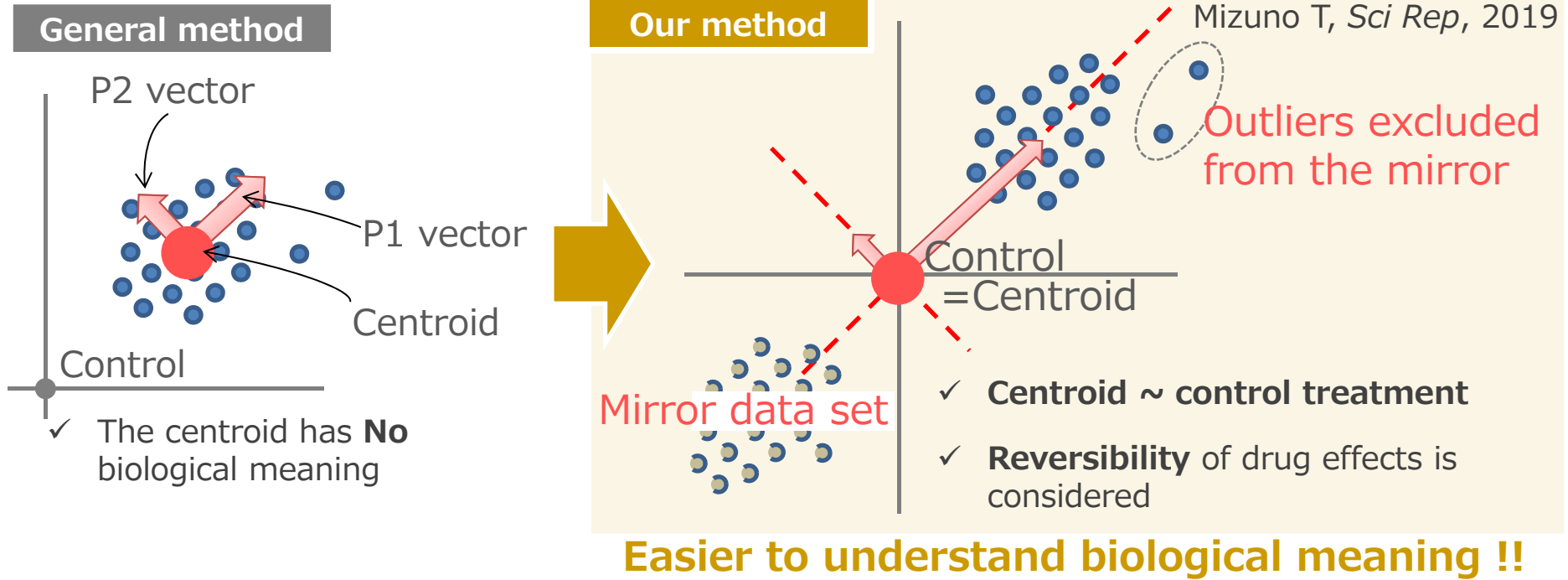
Kosaka, *Can Sci*, 2013

Some of drug repositioning may be called **drug redevelopment**

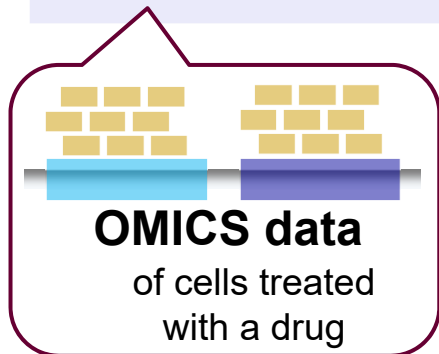
How to uncover unrecognized aspects?



Orthogonal Linear Separation Analysis (OLSA)



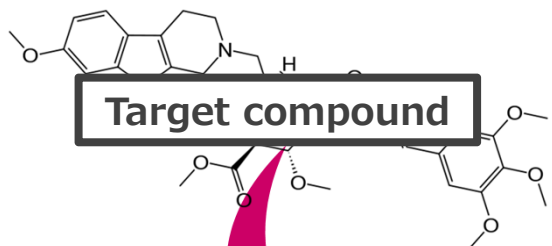
$$\underline{Data}_i = \underline{Score}_{i1} \underline{P1V} + \underline{Score}_{i2} \underline{P2V} + \dots + \underline{Score}_{ik} \underline{PkV} + \varepsilon_i$$



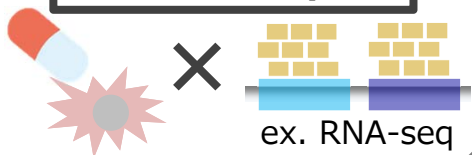
Response score
reflecting the degree of each decomposed effect (a scalar)

Gene 1	0.001	Response vector reflecting a decomposed effect
Gene 2	0.012	
⋮	⋮	
Gene 11911	0.271	

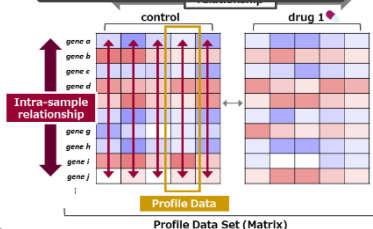
Scheme



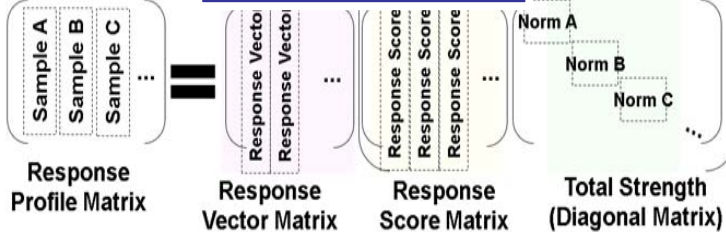
Omics analysis



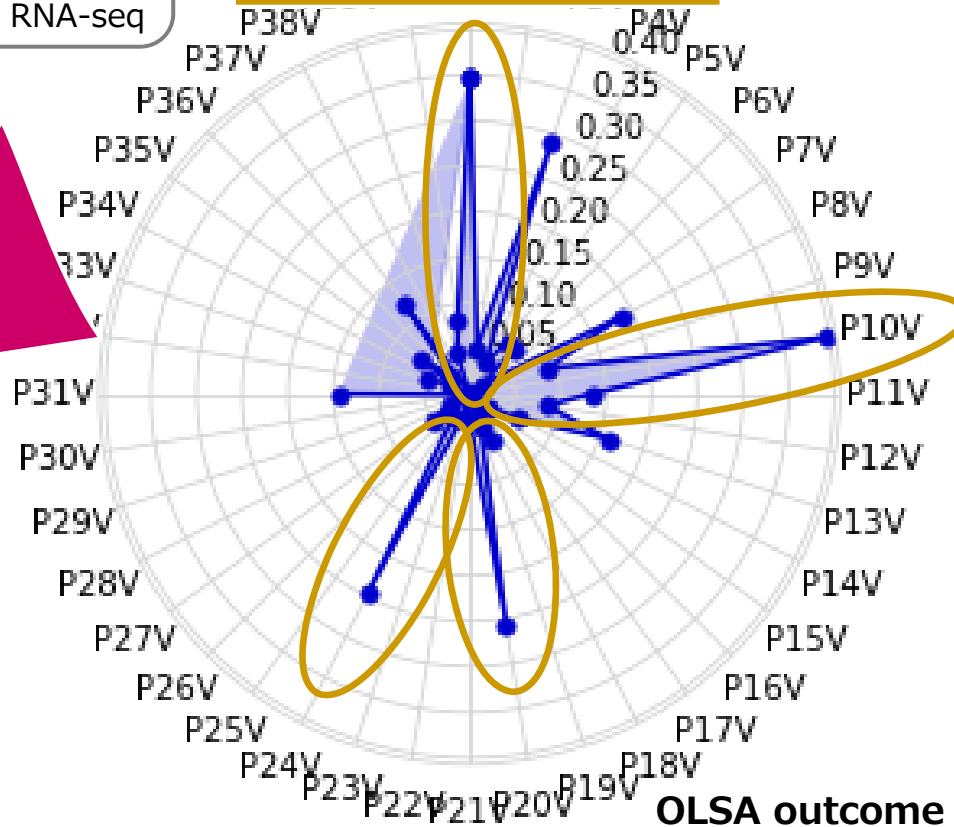
Profile data



OLSA



Decomposed Effects



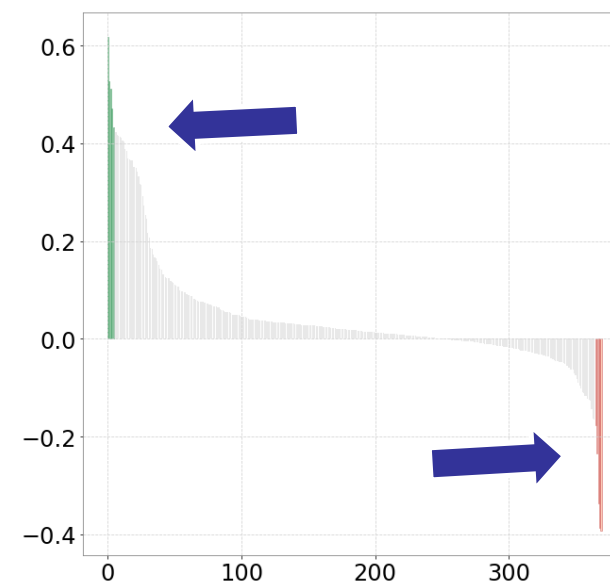
Direction of Scores

*microarray data of MCF7 cells treated with ~300 small compounds (<http://www.ilincs.org/ilincs/signatures/main>)

Factor	Contribution	Estimated Basic Effect	GO
7	1.70%	ER activation	system development (GO:0048731), etc

List of Scores in Training Data

Rank	Perturbagen	Dose (μM)	Score	Type
1	estradiol	0.01, 0.1	0.618	Estrogen
2	equilin	15	0.527	Estrogenic steroid
3	estradiol	0.01	0.512	Estrogen
4	estradiol	14.6	0.471	Estrogen
5	estradiol	0.1	0.432	Estrogen
⋮	⋮	⋮	⋮	
366	tamoxifen	7	-0.177	Estrogen antagonist
367	raloxifene	7.8	-0.234	Estrogen antagonist
368	fulvestrant	1	-0.337	Estrogen antagonist
369	fulvestrant	0.01	-0.387	Estrogen antagonist
370	fulvestrant	0.01, 1	-0.392	Estrogen antagonist



Topic

1. 化学物質が持つ作用のバイアスのない変換

- ✓ ハンドリングが容易な二次元電気泳動装置の開発

2. 教師無し解析による化学物質の作用分離

- ✓ 化学物質の作用分離解析手法の開発

3. 何ができるか？

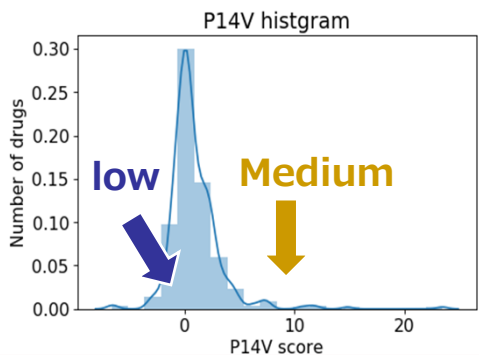
- ✓ 潜在的な毒性の検出
- ✓ 複合的な作用の分離

4. まとめ



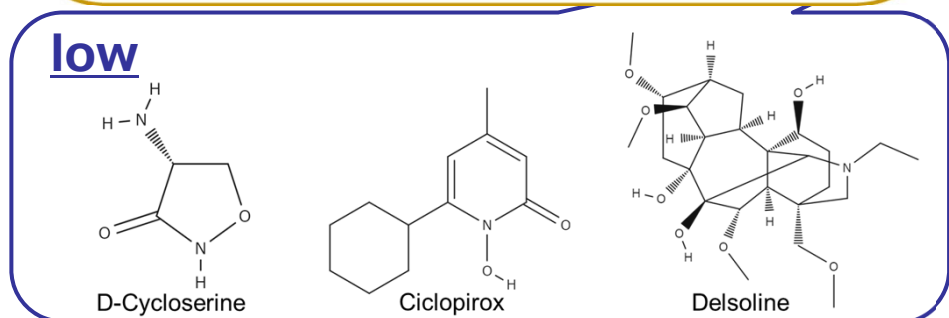
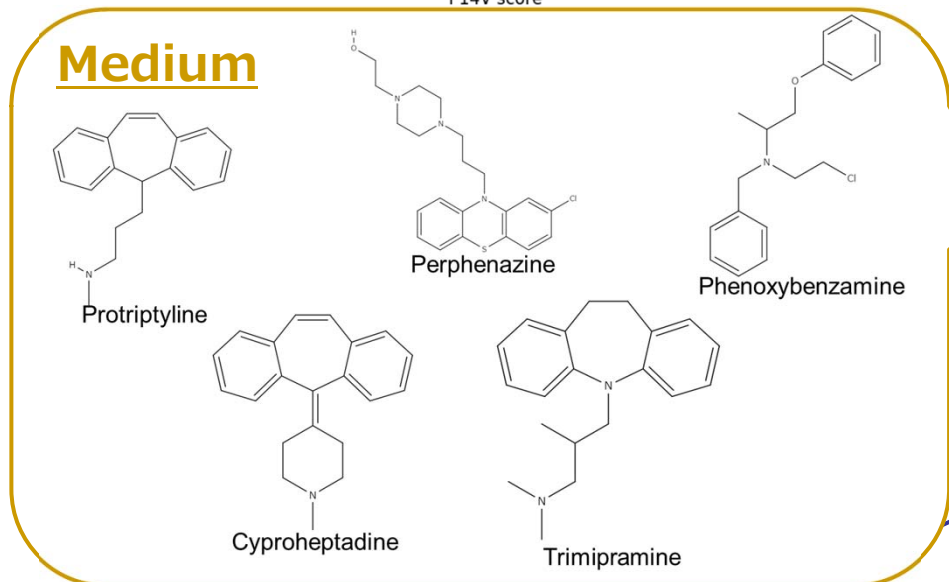
潜在的なERストレス誘導能の検出

*microarray data of MCF7 cells treated with ~300 small compounds (<http://www.ilincs.org/ilincs/signatures/main>)



Compound List Sorted with P14V Scores

Rank	perturbagen	Dose (μM)	P14V Score	Reputed (PMID)	FDA approval	remarks
1	thapsigargin	0.1	23.43	25126734		
2	ciclosporin	3.4	14.81	28324237		Control
3	ionomycin	2	11.98	7876163		
4	ciclosporin	1	11.43	28324237		
5	geldanamycin	1	10.64	9428803		
6	thioguanosine	12.6	7.66	no		
7	thiostrepton	2.4	7.49	24952196		
8	tanespimycin	1	7.34	25126734		
⋮	⋮	⋮	⋮	⋮	⋮	⋮
15	protriptyline	13.4	5.01	no	yes	candidate
16	mometasone	7.6	4.75	no	yes	Plaster
21	perphenazine	10	4.61	no	yes	candidate
21	phenoxybenzamine	11.8	4.25	no	yes	candidate
33	cyproheptadine	12.4	3.38	no	yes	candidate
34	trimipramine	9.8	3.32	no	yes	candidate
⋮	⋮	⋮	⋮	⋮	⋮	⋮
241	delsoline	8.6	-0.0089	no		negative
242	ciclopirox	15	-0.0130	27757583		negative
243	cycloserine	39.2	-0.0131	no		negative

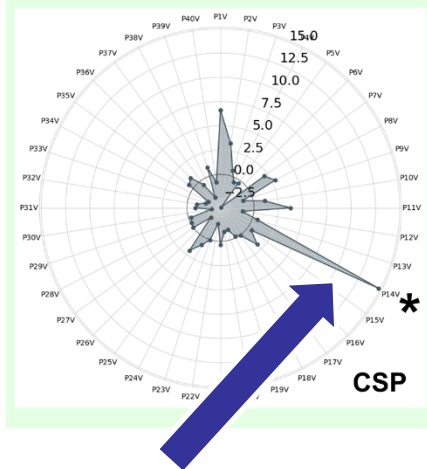
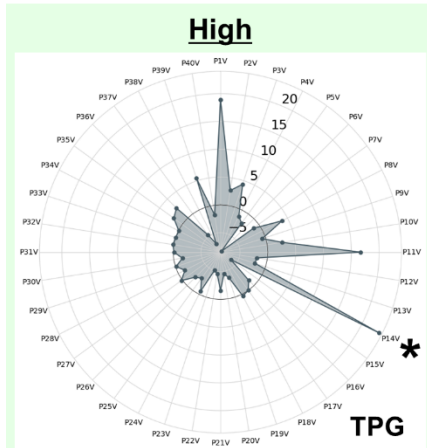


Criteria for Candidates

- ✓ Medium score
- ✓ Approved by FDA
- ✓ No reports in PubMed
- ✓ p.o. drug

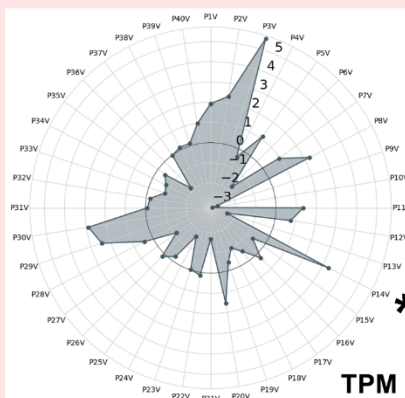
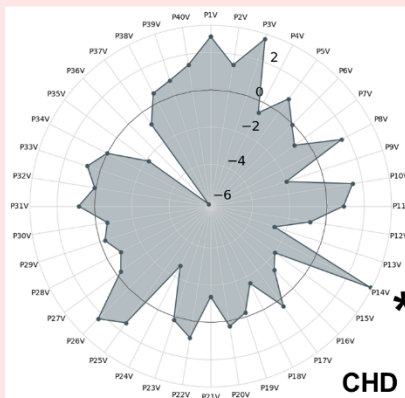
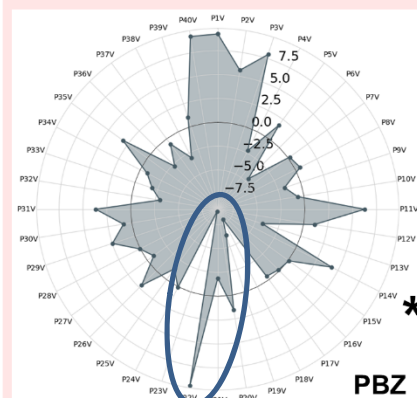
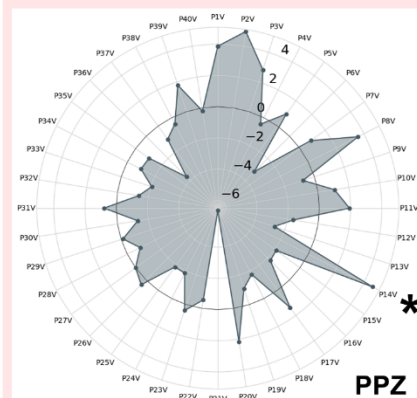
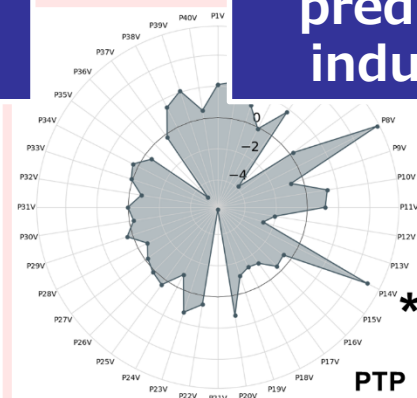
潜在的なERストレス誘導能の検出

well-known ER stress inducers

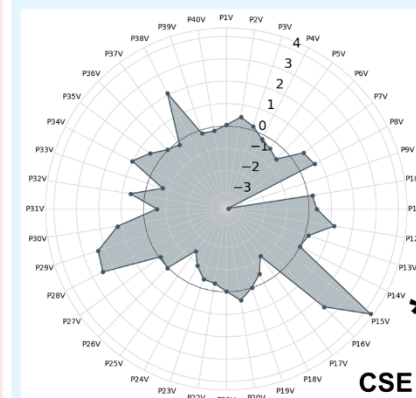
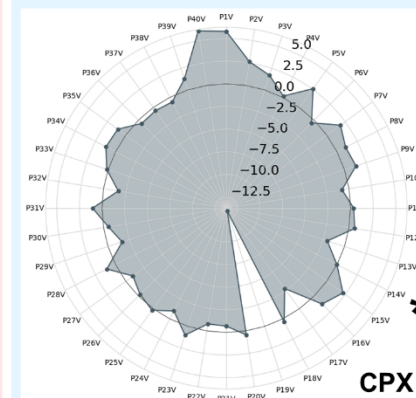
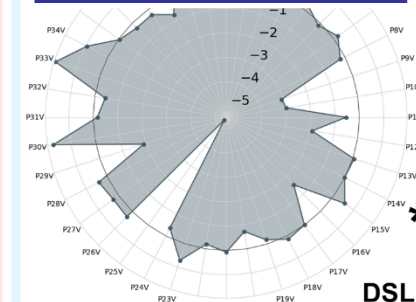


*ER stress vector

predicted inducers

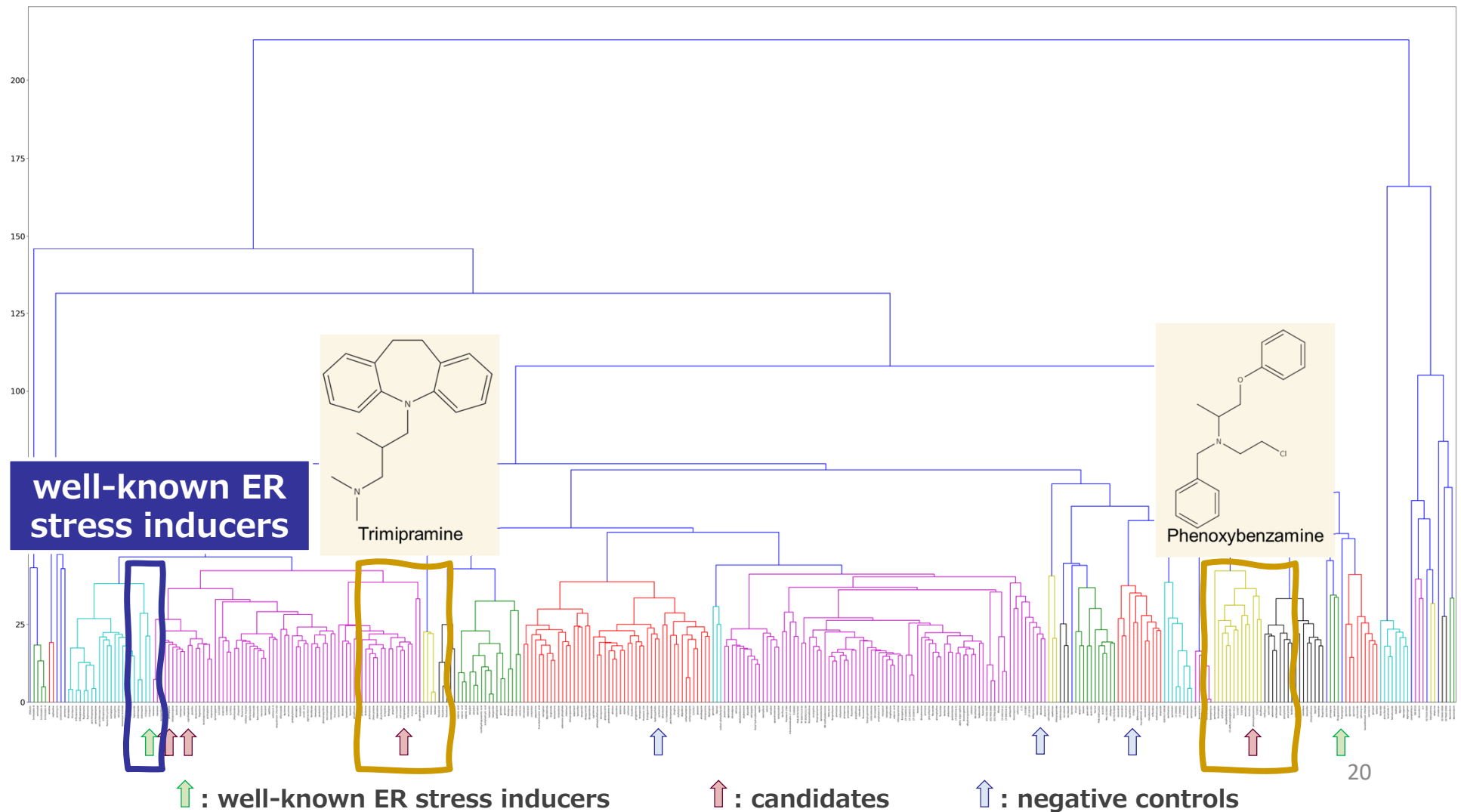


predicted negative



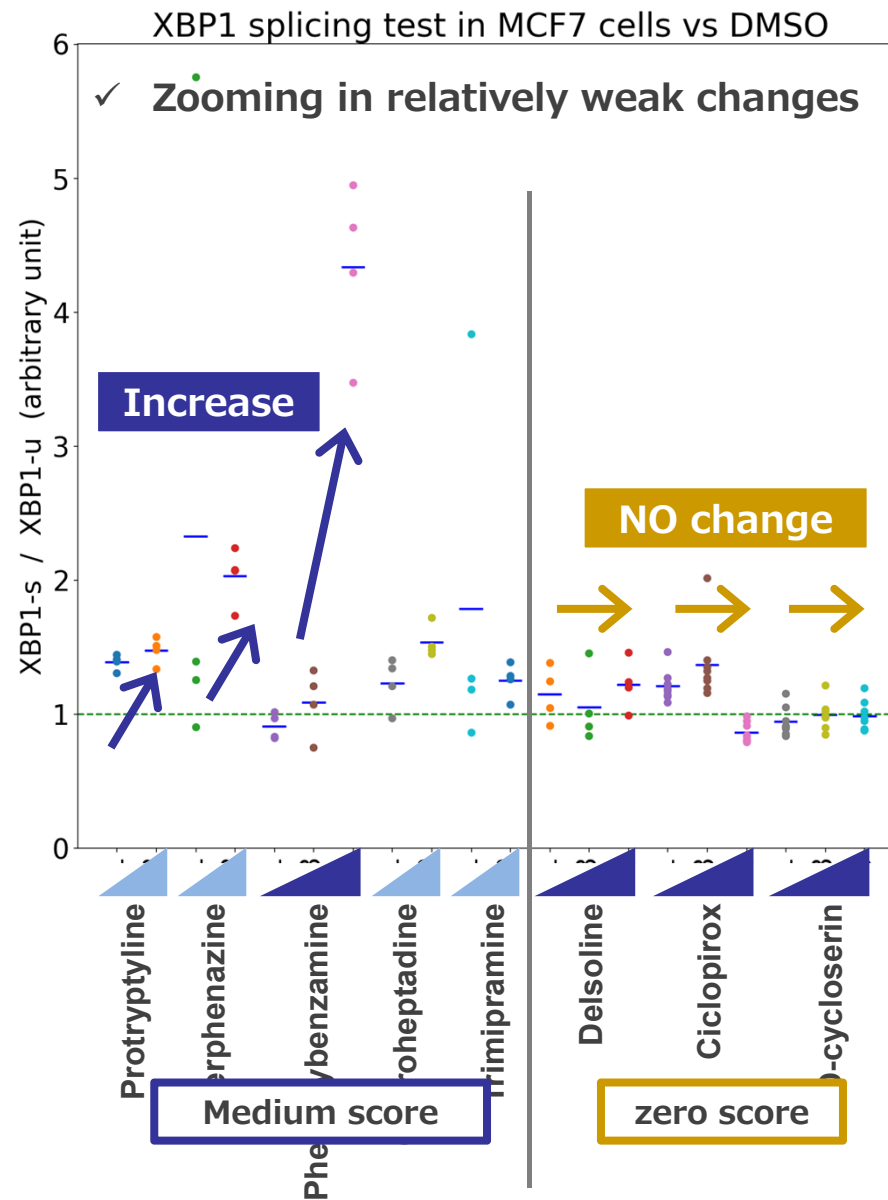
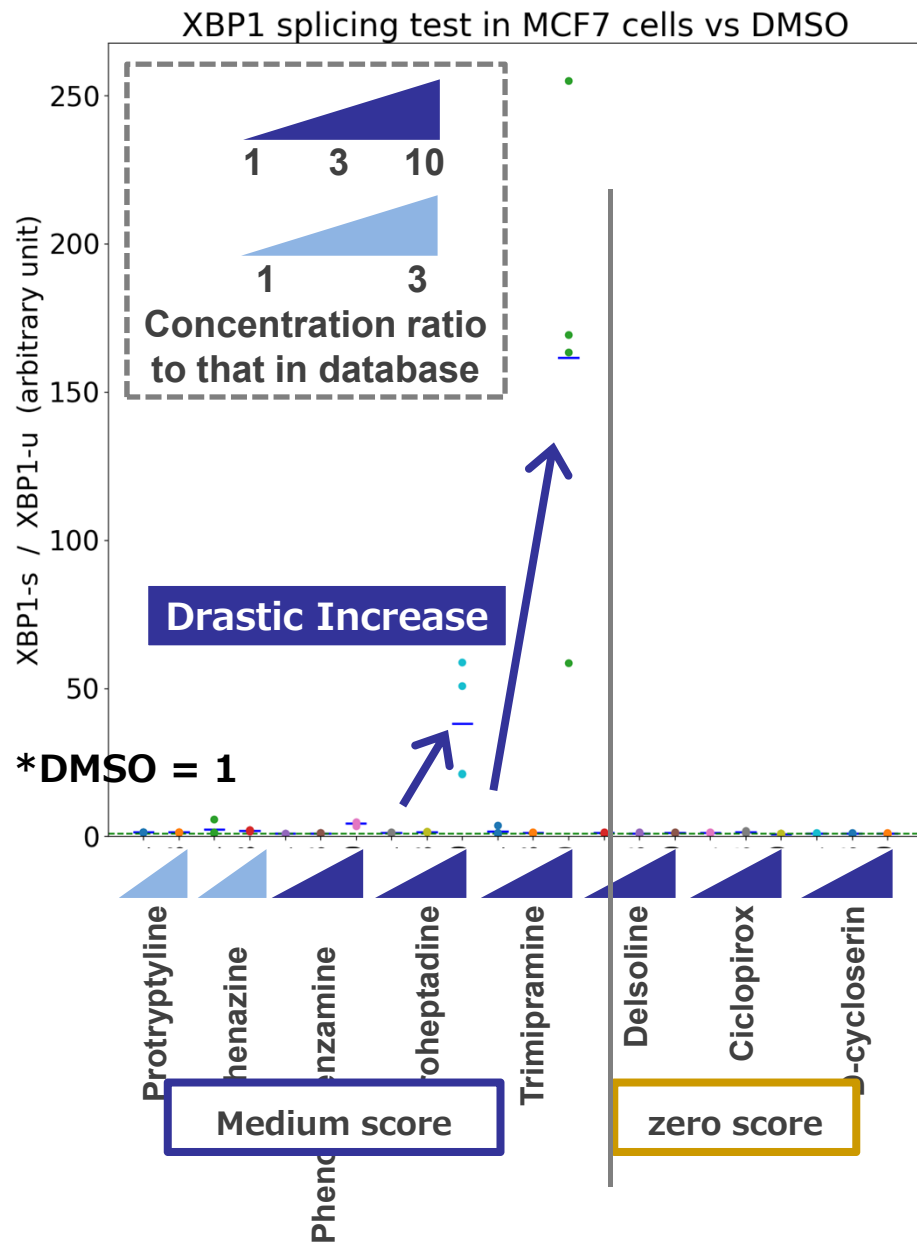
潜在的なERストレス誘導能の検出

Two of five candidates were clustered in clusters far from the well-known ER stress inducer cluster.



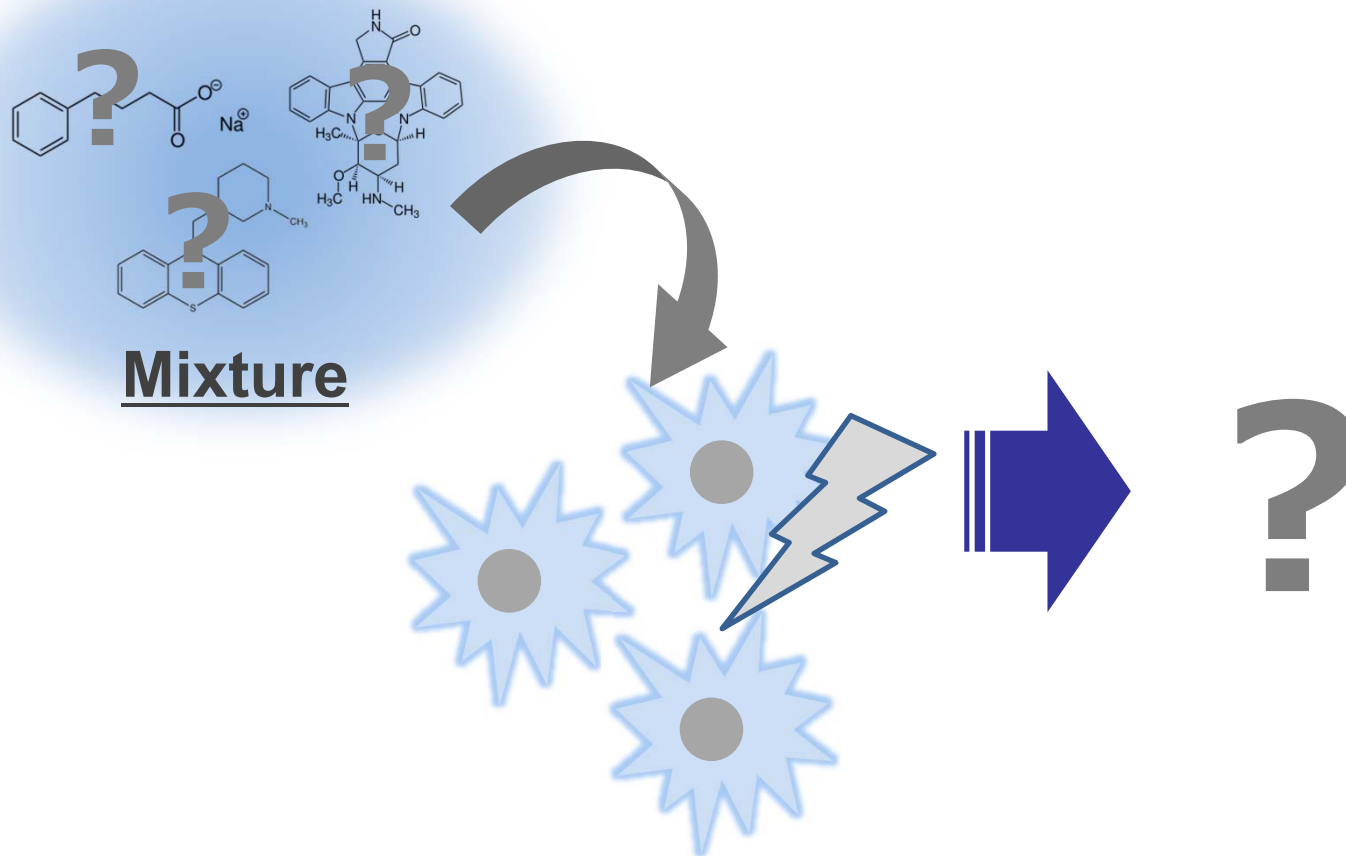
潜在的なERストレス誘導能の検出

Morita K, *Sci Rep*, 2020



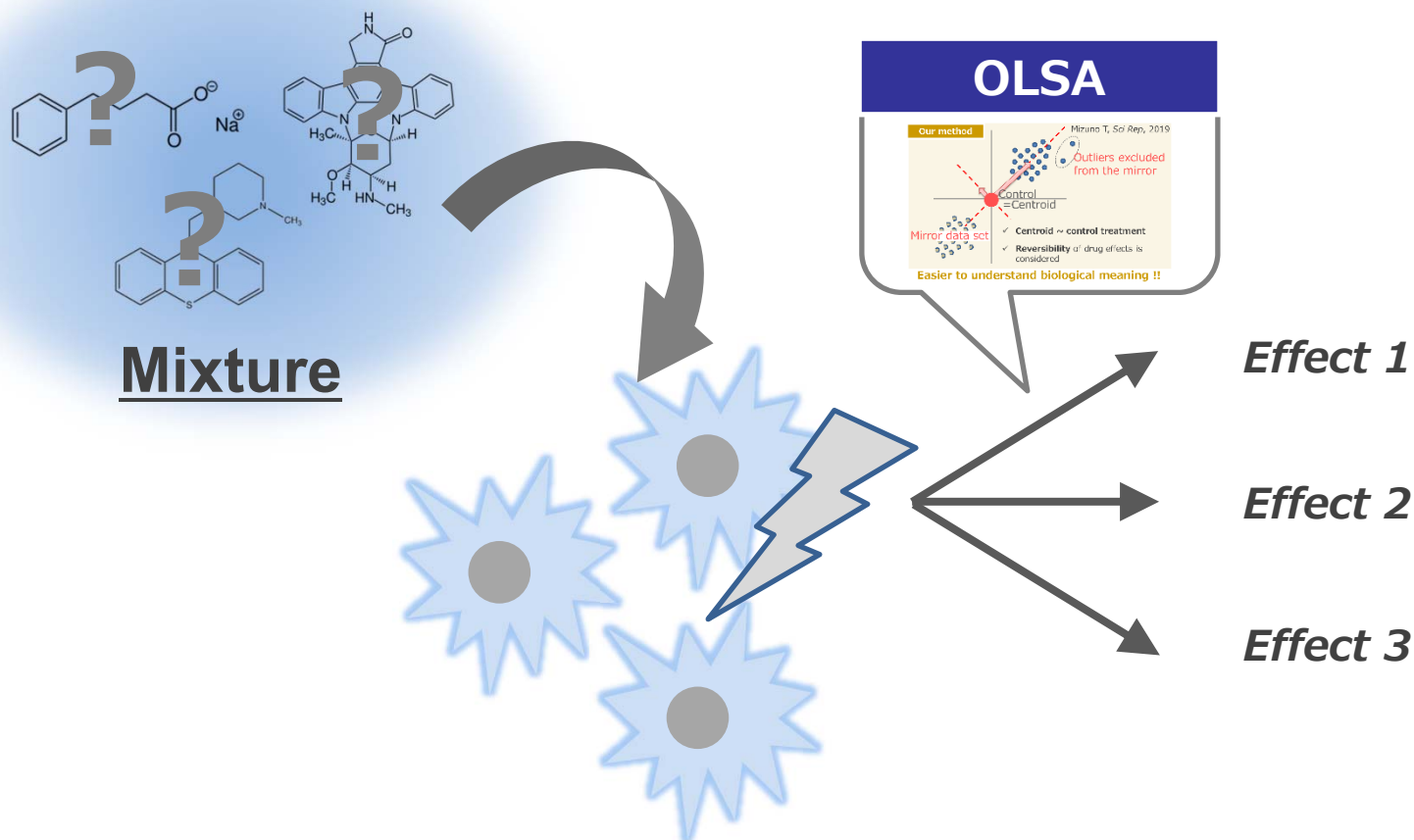
複合的な作用の分離

- ✓ We often want to know what effects a **mixture** has
 - ✓ Ex) Development of **Detergents, Natural Products, etc.**



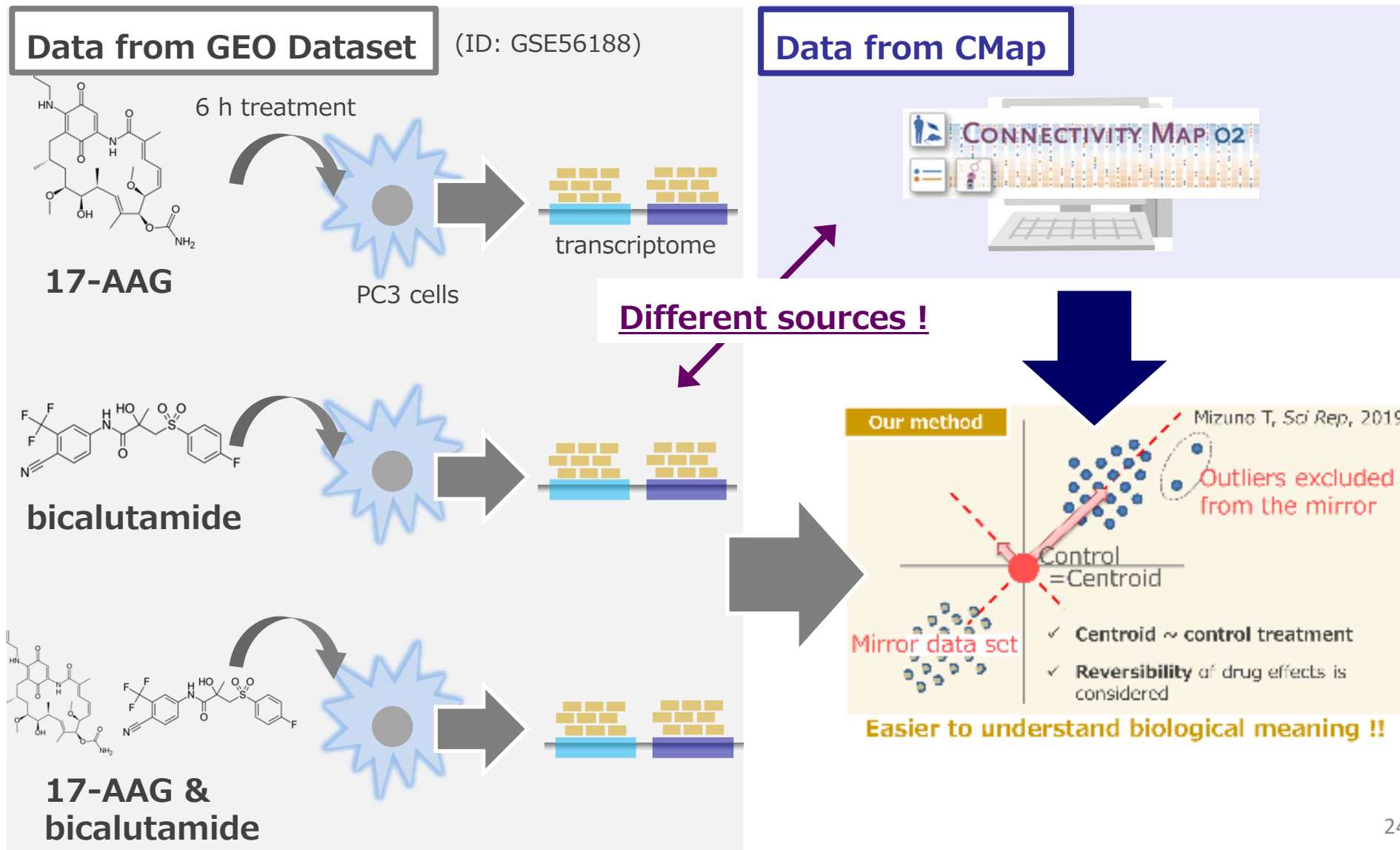
複合的な作用の分離

✓ Decomposition Approach May Be Useful in the Situation



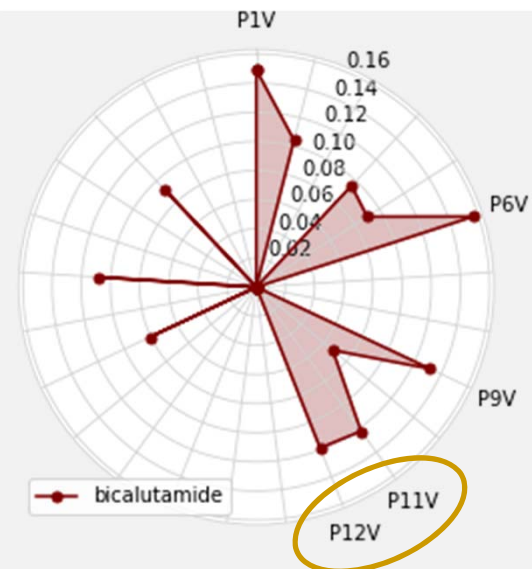
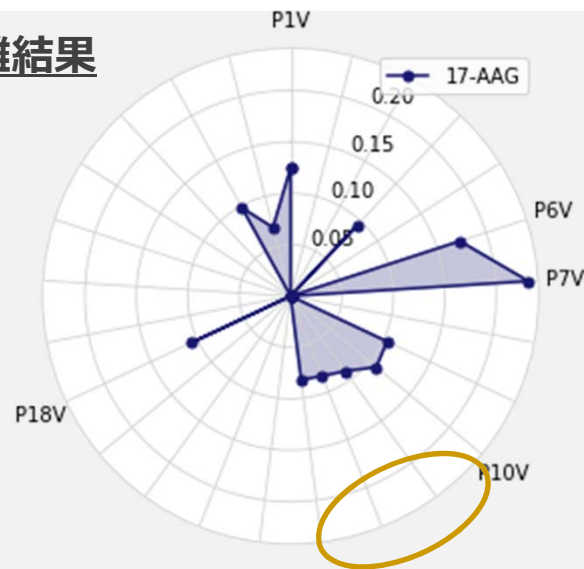
test case

- ✓ Whether data derived from a mixture is explained by data of single treatment?

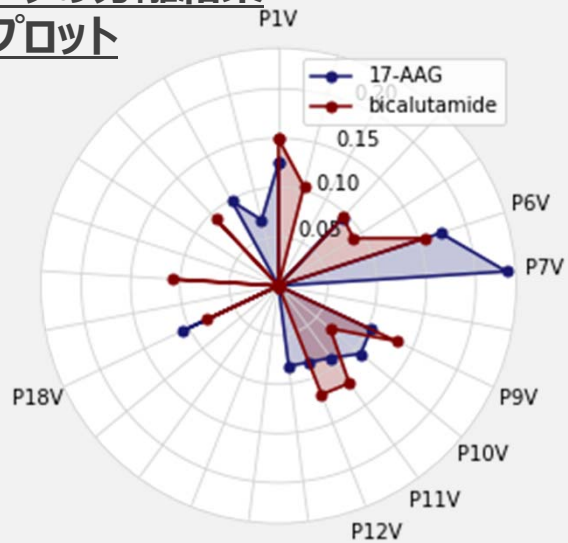


複合的な作用の分離

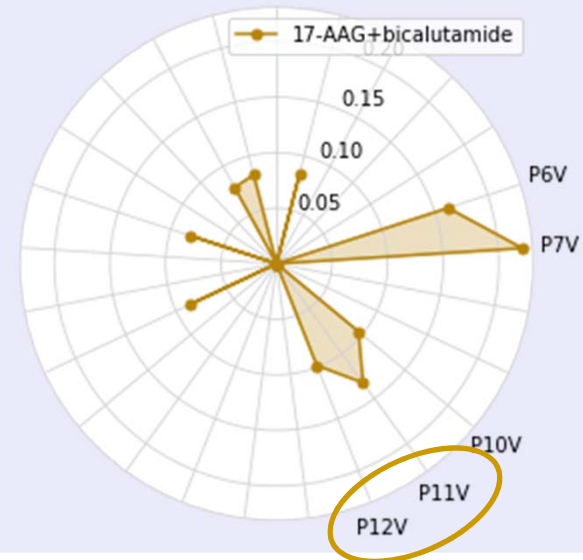
単剤データの分離結果
の単独プロット



単剤データの分離結果
の同時プロット

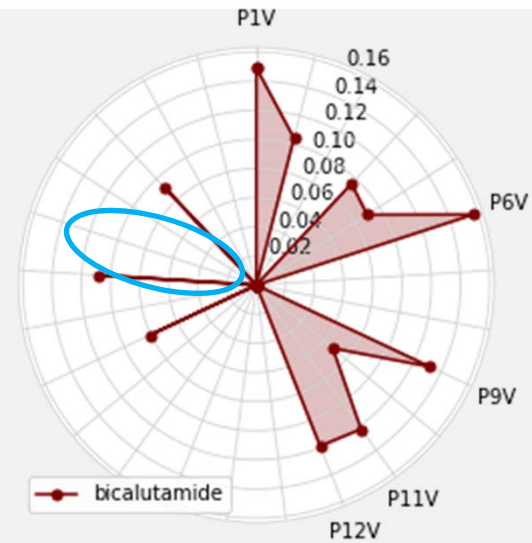
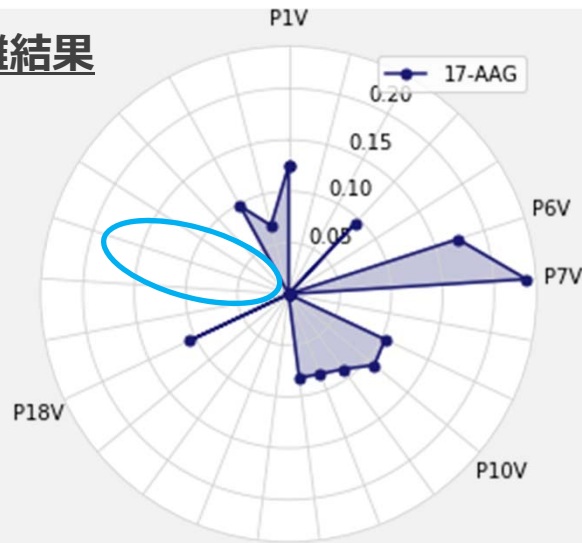


混合物データの分離結果のプロット

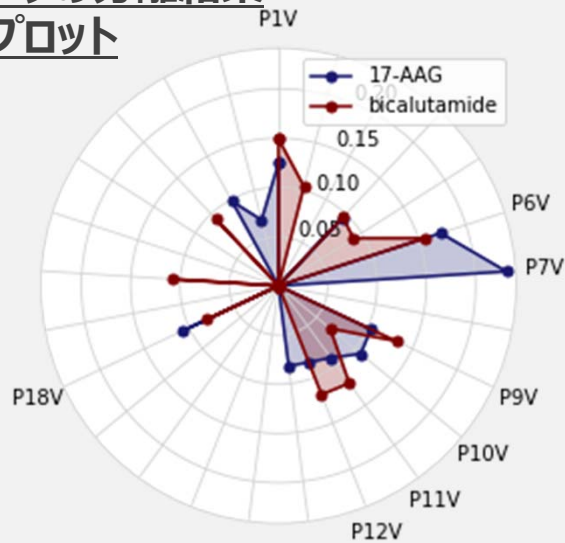


相乗効果？

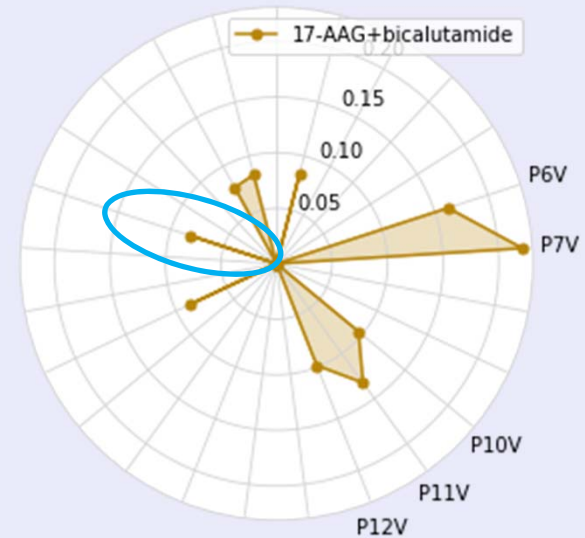
単剤データの分離結果の単独プロット



単剤データの分離結果の同時プロット



混合物データの分離結果のプロット



Topic

1. 化学物質が持つ作用のバイアスのない変換

- ✓ ハンドリングが容易な二次元電気泳動装置の開発

2. 教師無し解析による化学物質の作用分離

- ✓ 化学物質の作用分離解析手法の開発

3. 何ができるか？

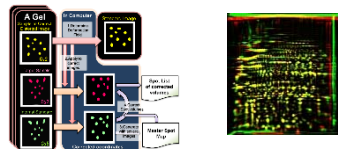
- ✓ 潜在的な作用の検出
- ✓ 複合的な作用の分離

4. まとめ

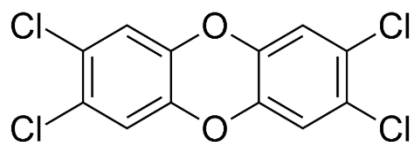
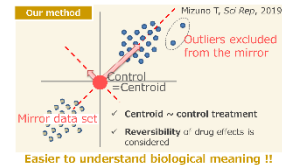


Summary

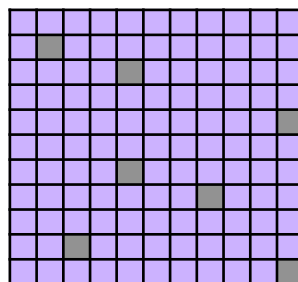
バイアスのない変換



教師なし解析

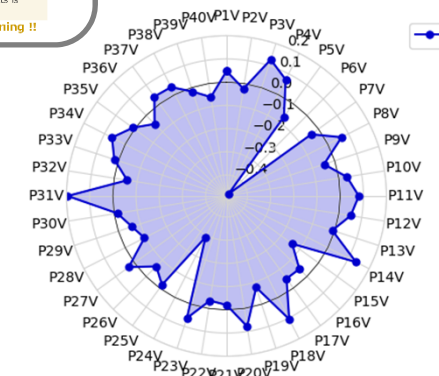


生物学的情報



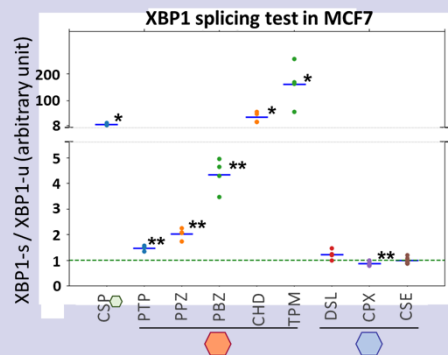
数値的情報

= プロファイルデータ

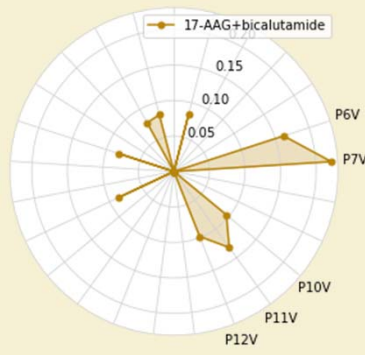


化学物質の作用分離

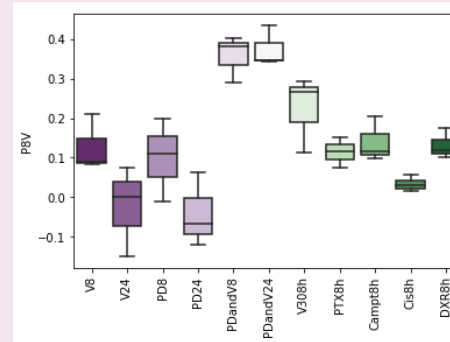
① (未知の作用も含めた) 恣意性のない作用評価



② 複合的な作用の評価

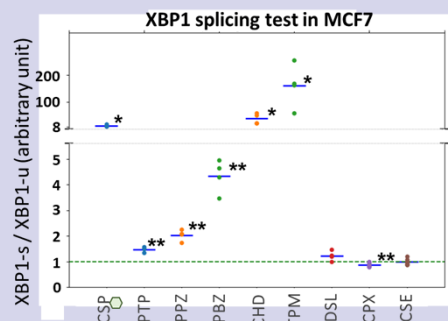


③ 相乗効果の評価



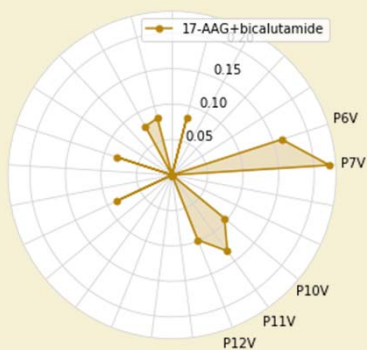
Summary

①(未知の作用も含めた)恣意性のない作用評価



- ✓ 化学物質の**潜在的な毒性**に対する**アラート**
- ✓ (逆に検出されない場合, 想定外の作用が見出される可能性が少ないことの保証?)

②複合的な作用の評価

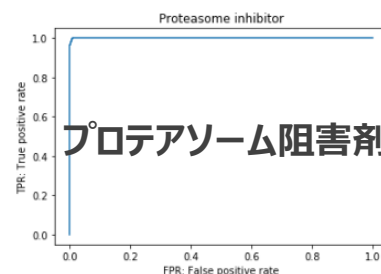


- ✓ **混合物**や**複合曝露**した際の**作用の多様性・強度**を, 単独投与と同じ評価指標で**評価可能**

Future Perspective

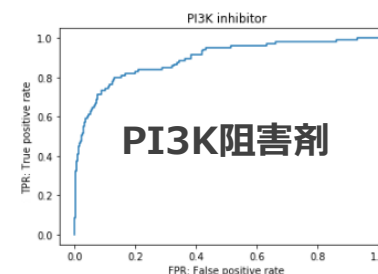


➔ Obtain data and Predict drug label based on transcriptome

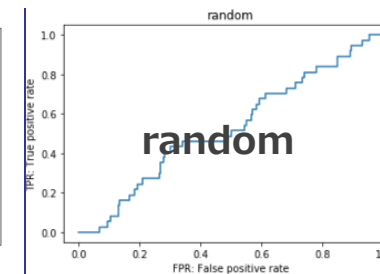


ROC-AUC

mean **0.996**,
std 0.004



mean **0.909**,
std 0.010



mean 0.480
std 0.044

- ✓ Label prediction (= catching main effect) is OK

How about **sub-effects**?



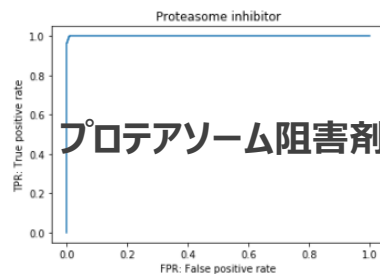
On going

- ✓ Utilization of OLSA
- ✓ Establishment novel decomposition profile data analysis for LINCS data
- ✓ Annotation by biological knowledge

Future Perspective

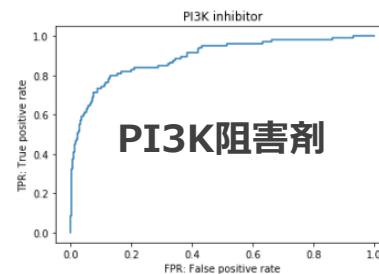


➔ Obtain data and Predict drug label based on transcriptome

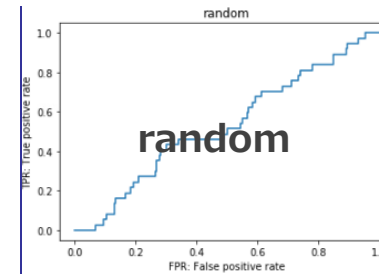


ROC-AUC

mean **0.996**,
std 0.004



mean **0.909**,
std 0.010



mean 0.480
std 0.044

✓ **in vitro** prediction is OK

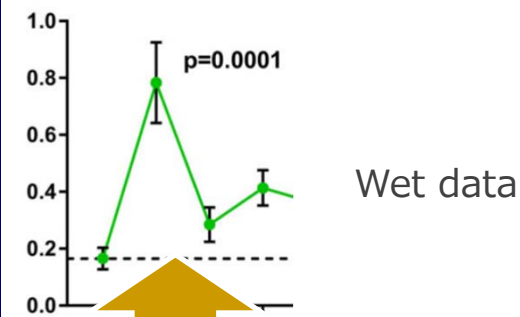
How about **in vivo/human**



Utilize information of the **bridging**

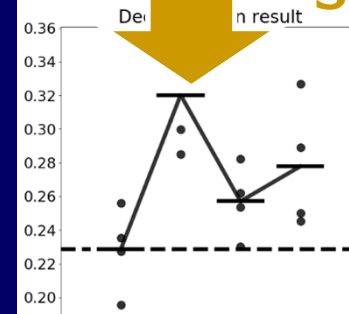
- ✓ immune cell population
- ✓ kinetics

Ex. Monocyte population change after APAP injection



Wet data

Similar !



Predicted data by our method

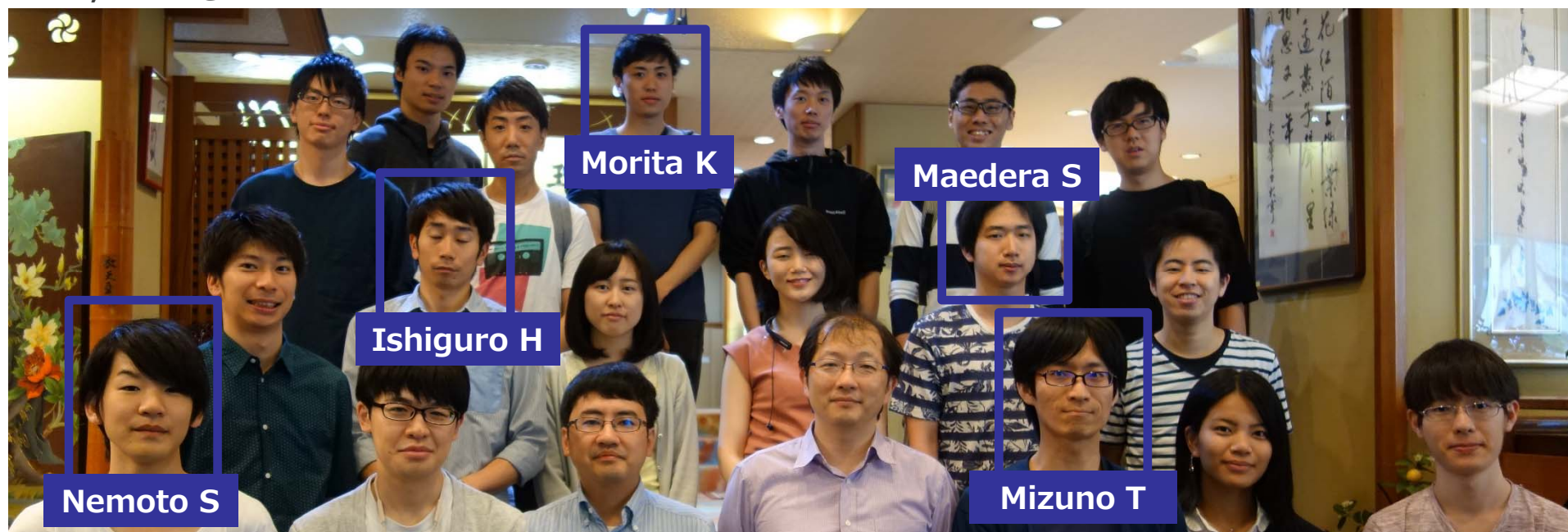
Acknowledgement

Financial Supports

- Long-range Research Initiative (Japan Chemical Industry Association)
- Grant-in-Aid for Challenging Exploratory Research (JSPS KAKENHI)
- Grant-in-Aid for Young Scientists (B)
- Mochida Memorial Foundation for Medical and Pharmaceutical Research



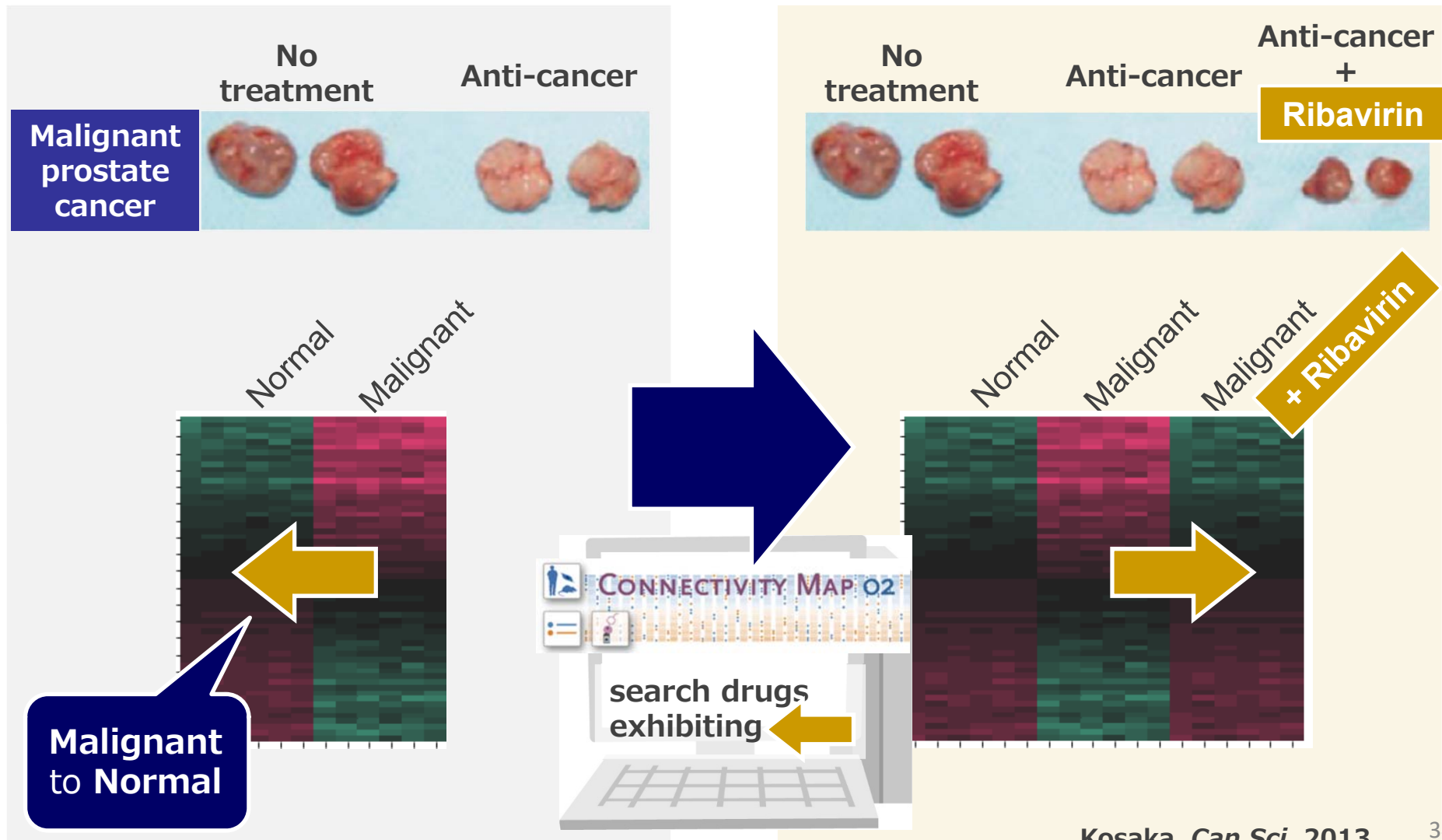
Jun, 2019@Izu



Appendix

Unrecognized aspects of chemicals

Ribavirin, an **anti-virus**, converts the malignant prostate cancer to the normal one



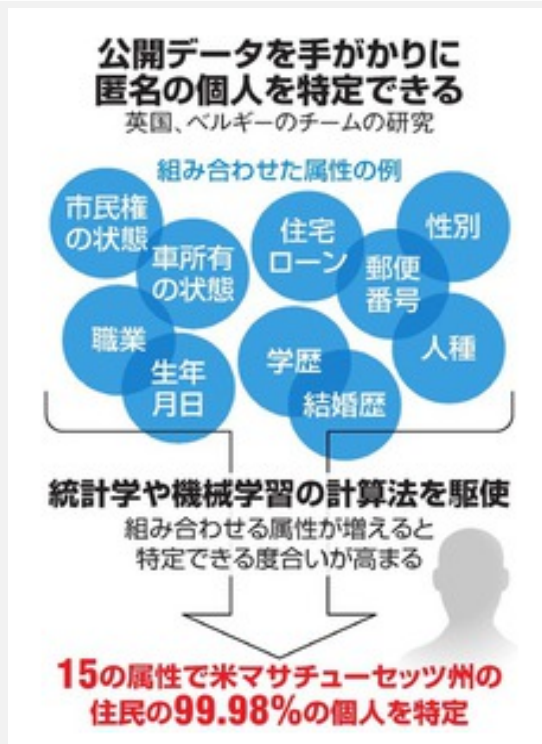
一般社会におけるプロフィールデータ

世の中でデータと呼ばれる多変量は大体該当

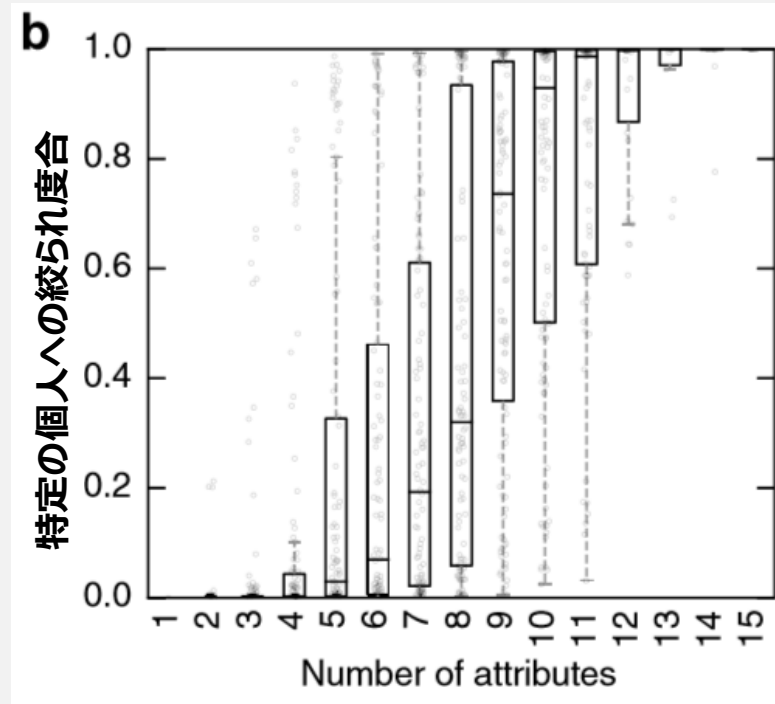
■ 活用例) 現在の個人情報匿名化技術の危うさ

- ✓ 複数の匿名化された個人情報から15の変数を各々集めると、極めて高い精度で一人に定まる
→ 匿名化された個人情報の再連結が可能

SNS, 保険会社, etc.が匿名化した一部を公開



2019/8/11 朝日新聞デジタル

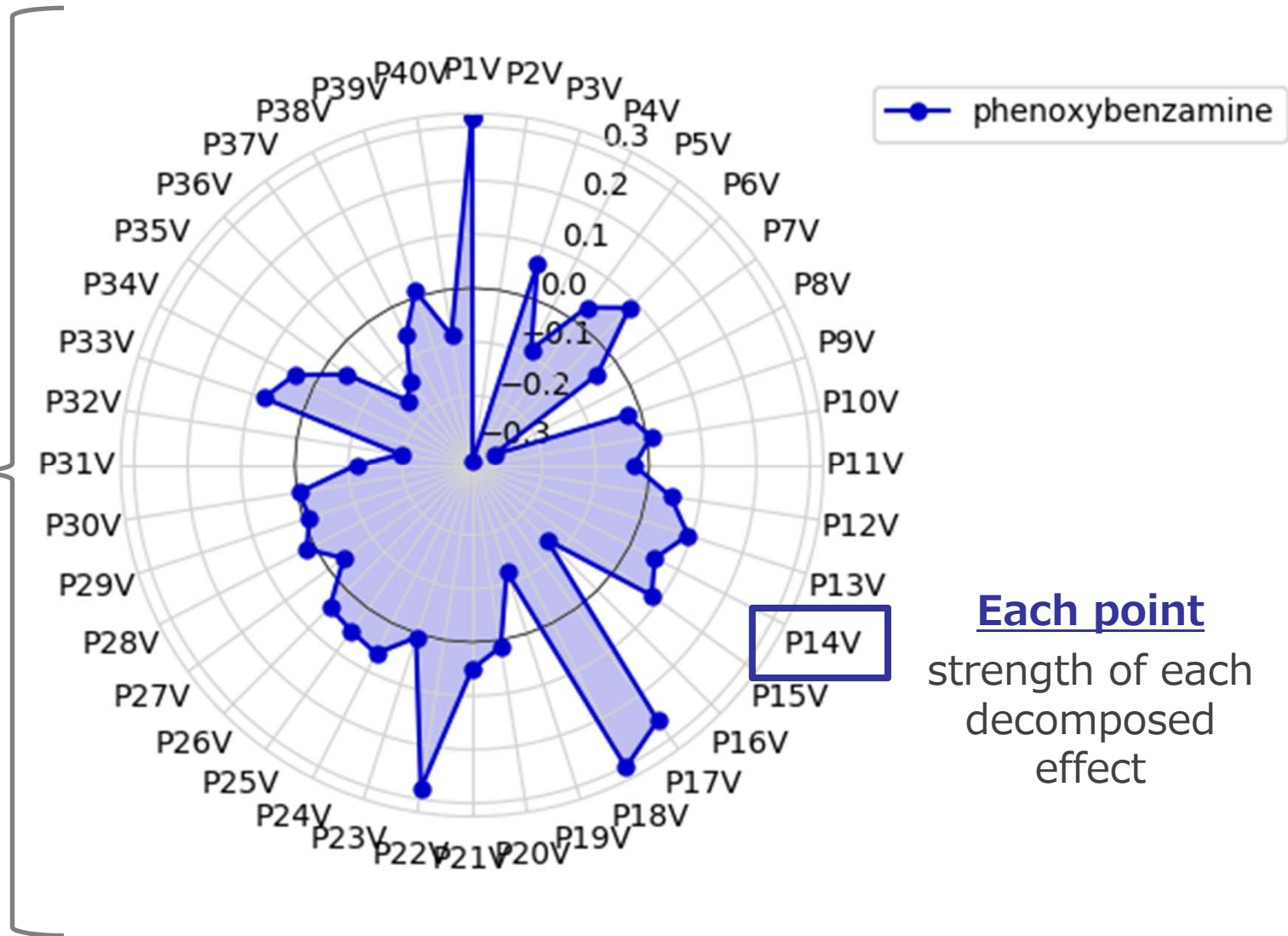


Rocher L, 2019, *Nat. Commun*

Visualization of Outcome

Radar-chart

Decomposed effects of a drug



Each point
strength of each
decomposed
effect

(Exploratory) Factor Analysis

$$X = FL^T + E$$

Factor

Variable

Uniqueness

