

**Metabonomics in the study of  
Traditional Chinese Medicine**

**College of Medicine, Kew**

**Peter Hylands**

**6 July 2011**

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**TRADITIONAL CHINESE MEDICINE WEEK**  
**中医药周**  
to 2 August 2008

15th Anniversary of the Association of Traditional Chinese Medicine of the Royal College of Physicians of the United Kingdom and the Chinese Embassy in the United Kingdom (China)

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Traditional Chinese medicine seeks to adjust the circulation of qi (metabolic energy flow) in the body using a variety of therapeutic techniques

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Some of these techniques include

- Special diets
- Physical training regimens (*qigong*, *tai chi chuan*, and other martial arts training)
- Massage
- Acupuncture
- Moxibustion
- Herbal medicines

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The Five elements:

Tree, traditionally **Wood**, (木, pinyin: *mù*)

**Fire** (火, pinyin: *huǒ*)

**Earth** (土, pinyin: *tǔ*)

**Metal** (金, pinyin: *jīn*)

**Water** (水, pinyin: *shuǐ*)

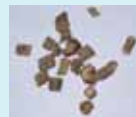
melon peel (300g),  
poria (300g),  
quince fruit (100g),  
perilla leaf (100g) and  
tangerine peel (100g)



An increasing number of trials:

### Effectiveness of a TCM preparation in cough treatment in uncomplicated upper respiratory tract infection

- Randomised, double blind, placebo-controlled, between groups study of 80 patients
- Average 4 days history of cough
- 5 days treatment, followed up for 6 days
- Evaluated by cough score



**Ledebouriella root**



**Fritillaria cirrhosa bulb**



**Citrus reticulata peel**





### Effectiveness of a TCM preparation in cough treatment in uncomplicated upper respiratory tract infection

- Randomised, double blind, placebo-controlled, between groups study of 80 patients
- Average 4 days history of cough
- 5 days treatment, followed up for 6 days
- Evaluated by cough score
- **No significant overall difference between groups**

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### Problems with conducting trials on TCM

#### The test material itself

Plant extracts (phytomedicines) are mixtures of many compounds

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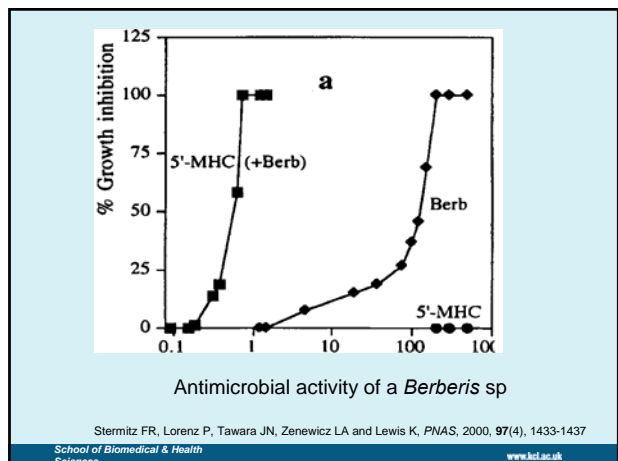
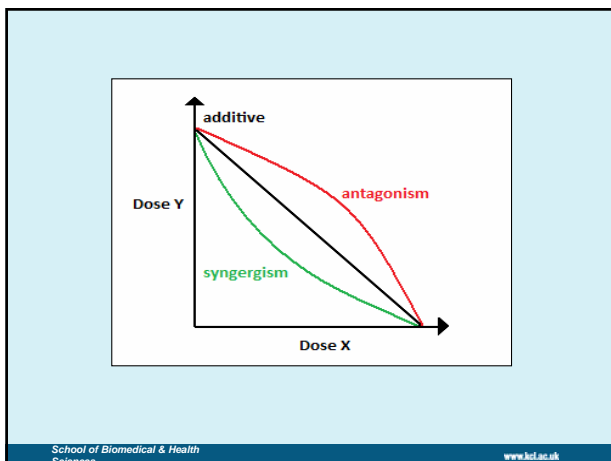
### Verification of synergy

Bioactivity-guided fractionation of a plant with reputed antimicrobial activity

*Berberis* species

Anti-infective modulators

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## Distinguishing feature of phytomedicines

**Extracts (mixtures of compounds) *not* single compounds**

Accepted conventional pharmaceutical methods not really applicable to plant extracts

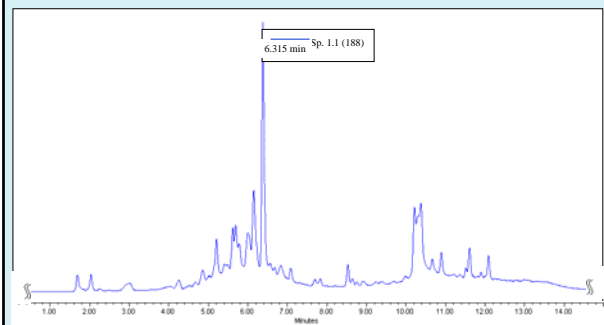
Nonetheless, standardisation often uses single actives or markers

This produces significant difficulties

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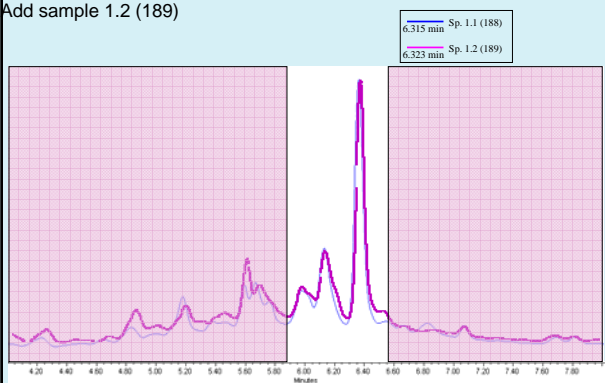
ONP-22 whole chromatogram



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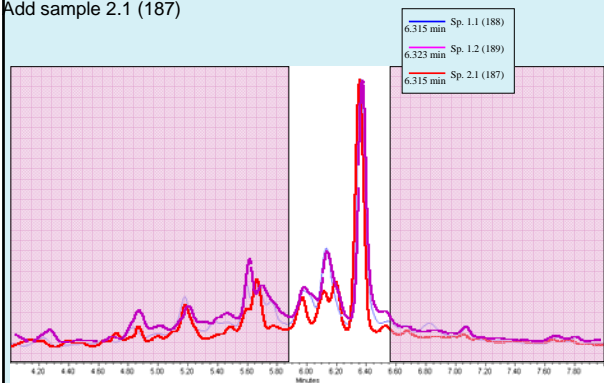
Add sample 1.2 (189)



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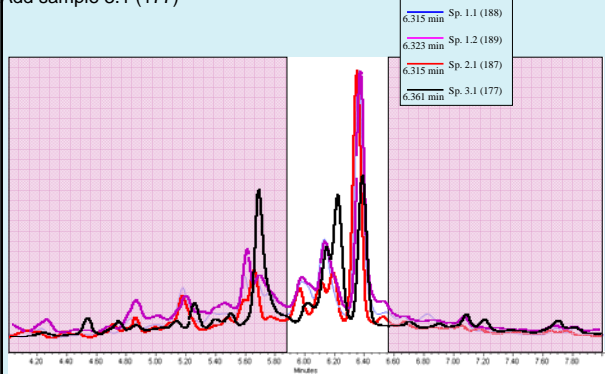
Add sample 2.1 (187)



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Add sample 3.1 (177)



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## Distinguishing feature of phytomedicines

**Extracts (mixtures of compounds) *not* single compounds**

**TCM products are mixtures of mixtures**

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# Metabolite profiling

## Metabonomics

## Metabolomics

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## What is metabolite profiling?

Investigating and describing genetic expression by **non-targeted** measurements of all metabolites in a biological sample

Profiling complex matrices such as *biofluids (plasma and urine)*

Biomarkers for metabolic studies and diagnosis  
environmental samples  
plant matrices (identification)

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## Tools available for metabolite profiling are several and can be hyphenated:

**Chromatographic**

- HPLC or GC
- GC-MS; HPLC-MS; HPLC-MS-MS
- Detection: selectivity
- Derivatization
- LC-UV-SPE-NMR-MS (cryogenic flow probe)

**Spectroscopic**

- ESI-MS
  - ionisation and fragmentation variability
- IR
  - data information limited
  - peak deconvolution algorithms necessary

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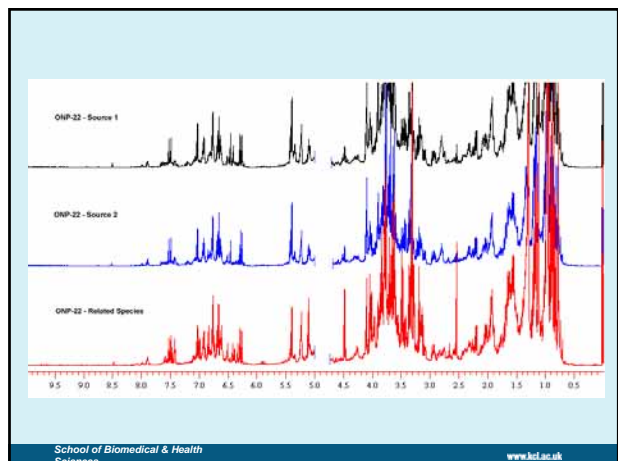
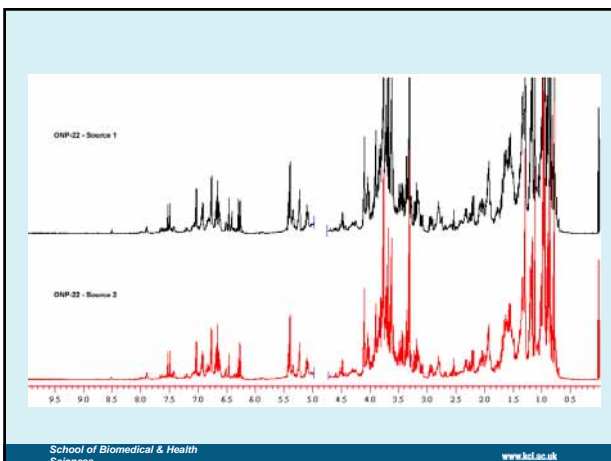
## High field <sup>1</sup>H nuclear magnetic resonance spectroscopy

Data rich fingerprint; requires

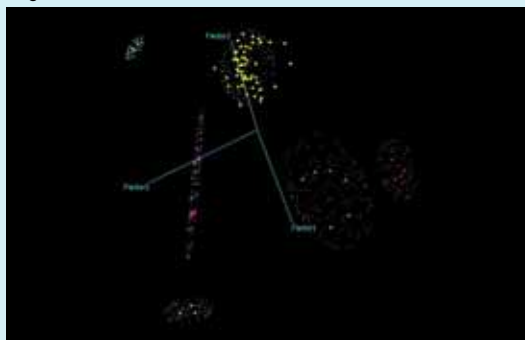
Data reduction/simplification

Statistical analysis:

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PCA – Intraspecific variation: target (field samples) and non-target accessions



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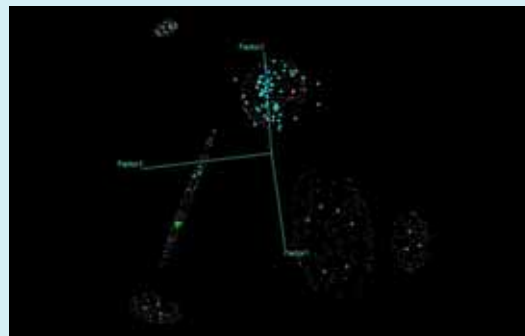
PCA – Intraspecific variation: target and non-target accessions + Year 1 Harvest



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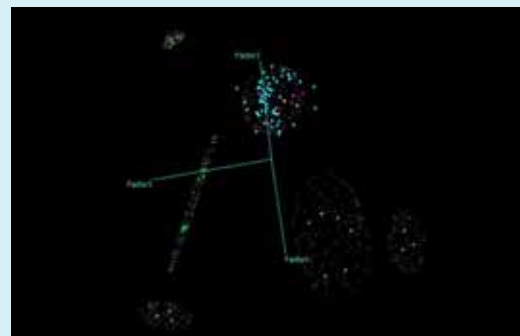
PCA – Intraspecific variation: target and non-target accessions + Year 2 Harvest



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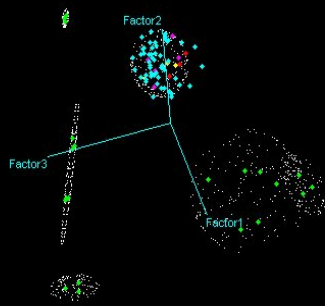
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PCA – Intraspecific variation: target and non-target accessions + Year 3 Harvest



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## Conclusion

'Fingerprinting' approaches already recognised by  
WHO  
Chinese State Food and Drug Authority

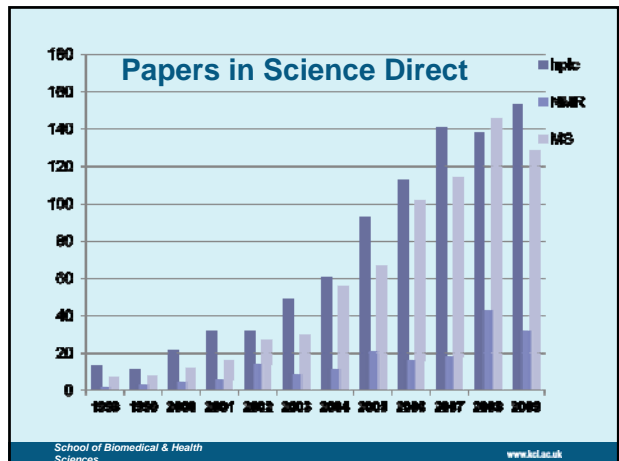
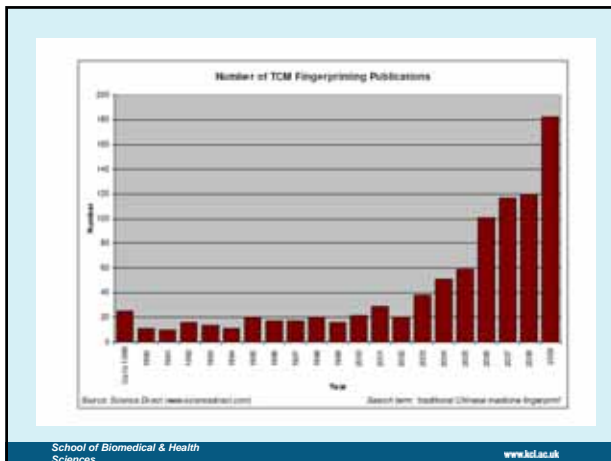
Only HPLC profiles considered

Reductive (not all compounds taken into account)  
Subjective in operation (impossible to set criteria for  
more than one peak)

NMR and PCA gives an approach to  
fuzzy fingerprints

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Application to metabolic studies

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Application of metabonomics to detect metabolic effects of plant products

High field nmr spectroscopy to analyse urine to investigate effects of chamomile tea ingestion

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Experimental design

Human volunteers

*Matricaria chamomilla* tea prepared from Roman chamomile flowers

Y Wang, H Tang, J K Nicholson, P J Hylands, J Sampson and E Holmes, *Journal of Agricultural and Food Chemistry*, 2005, 53(2), 191-196

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## Experimental design

Human volunteers

*Matricaria chamomilla* tea

- 5g dried flowers infused in 200ml hot water (10 minutes)
- Resultant tea drunk

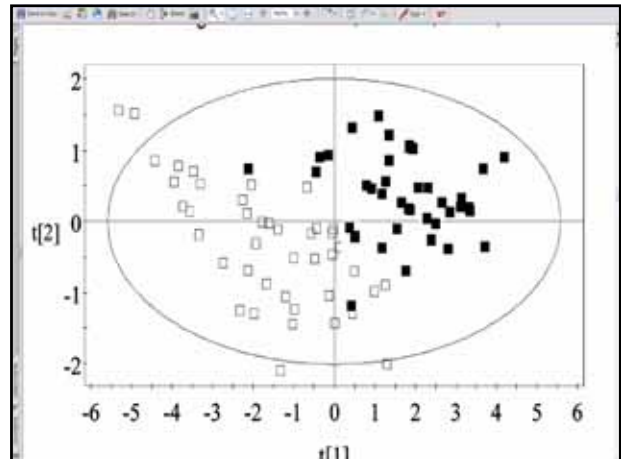
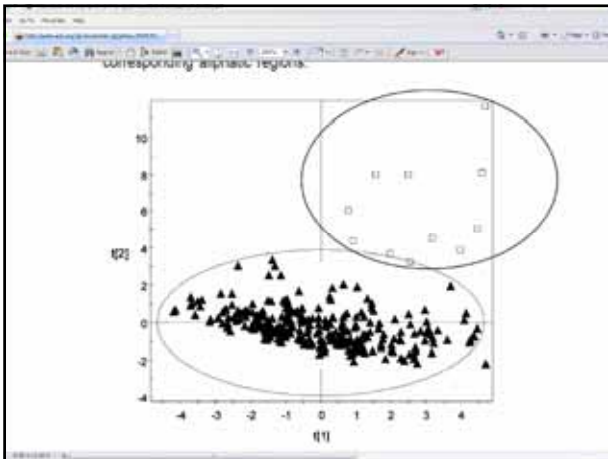
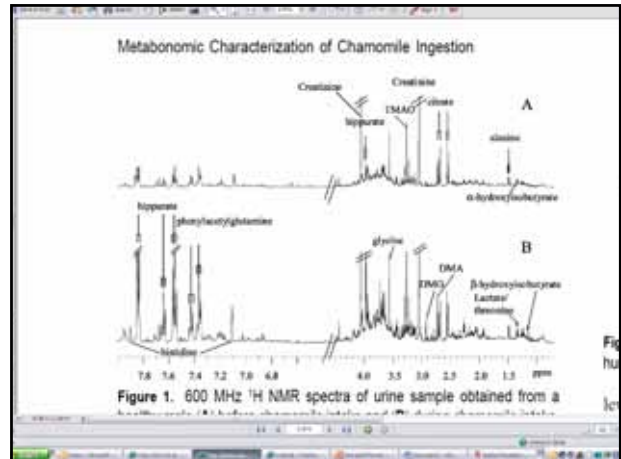
3 phases

- Pretreatment or control
- Treatment
- Post-treatment or washout

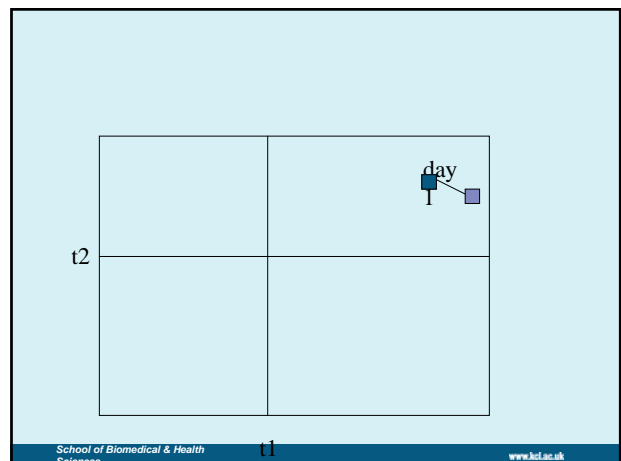
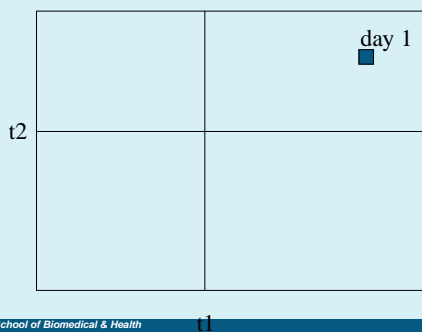
Urine collected 2 hours after dosing

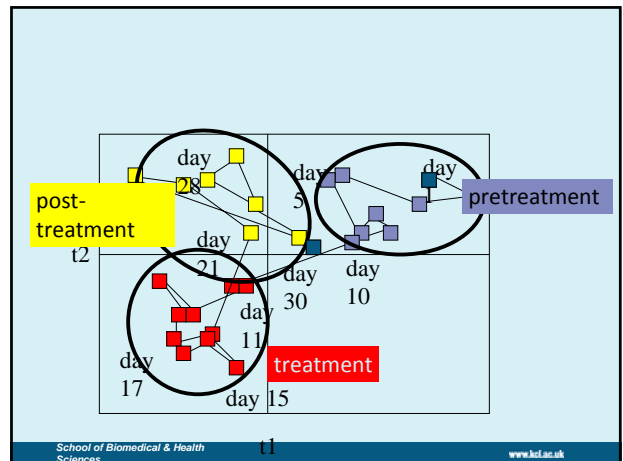
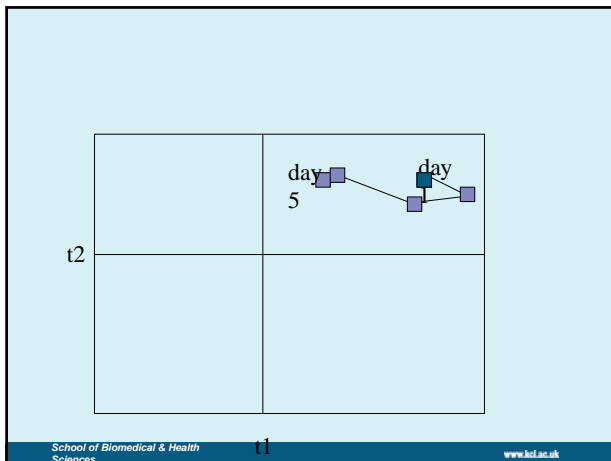
Urine examined by 600MHz nmr spectroscopy

Data analysed by partial least squares analysis



## Trajectory analysis of scores plot





Bioinformatics and Traditional Chinese Medicine

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### Traditional Chinese Medicine

TCM category	Western Equivalent	Signs and Symptoms
Tonify Yin	Endocrine agent, Antidiuretic, Antihypertensive, Anticholesterolaemic	Dizziness, tinnitus, weak lower back and knees, low-grade fever, menopausal symptoms, scanty dark urine, red dry tongue, thin pulse
Shen	Tranquillizer, Sedative, Nerve Tonic	Palpitations, anxiety, insomnia
Wind Cold	Diaphoretic, Antiviral, Antibacterial	Chills, headache, body & neck pain, no fever/mild fever
Heat (Qi)	Refrigerant, Antipyretic, Anti-inflammatory, Antimicrobial	High fever, irritability, thirst, delirium, certain skin diseases

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Drug discovery from TCM plants

Construction of Two Novel Databases:  
 Chinese Herbal Constituents Database (CHCD)  
 Bioactive Plant Compounds Database (BPCD)

T M Ehrman, D J Barlow and P J Hylands, *J Chemical Information & Molecular Modeling*, 2007, 47, 254-263

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### CHCD Database

	<b>Tribal name</b>	Ginsenoside Rg <sub>1</sub>	<b>Synonym</b>	Ginsenoside A <sub>6</sub> , Panaxoside A, Sanchinoside C <sub>1</sub>	<b>Systematic name</b>	Dammar-24-ene-1,6,12,20-tetraol; 6,20-Di-O-β-D-glucopyranoside	
	<b>Herb (genus)</b>	(1) Ren Shen, (2) San Qi	<b>Species</b>	(1) <i>Panax ginseng</i> , (2) <i>Panax notoginseng</i>	<b>Part of plant</b>	Root	
<b>CAS number</b>	22827-39-8	<b>Class</b>	Interposed	<b>Skeletal type</b>	Dammarane	<b>Chirality</b>	(1R,6S,12R,20S) or (7S,6S,12R,20S)
<b>Pharmacology</b>	Adaptogenic*, CNS stimulant*, Immunomodulator*, Tumour-inhibitory activity	<b>Toxicology</b>	LD <sub>50</sub> (mice, ip): 405 mg/kg, LD <sub>50</sub> (mice, ip): 1,600 mg/kg, RTECS No. LV957280	<b>References</b>	Sanda S et al. <i>Shoyabugaku Zasshi</i> , 1978, 22; 86 [P. notoginseng], Yahara S et al. <i>Chem. Pharm. Bull.</i> , 1976, 24: 2208; Yahara S, et al. <i>Chem. Pharm. Bull.</i> , 1979, 27: 88 [P. ginseng]. *Duke, J.A., <i>Biologically Active Phytochemicals and Their Activities</i> , CRC Press, 1992, p. 70.		

Entry for Ginsenoside Rg<sub>1</sub>

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## Phytochemical Informatics of TCM

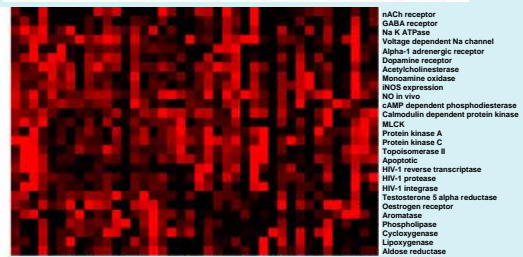
- Random forest models used to create TCM profiles
- Combined with Random forest models for target activities
  - **Predict activity** for drug discovery
- Analysed using Michael Eigen's **CLUSTER** program
- Visualized using Eigen's **TREEVIEW** program

T M Ehrman, D J Barlow and P J Hylands, *Journal of Chemical Information and Modeling*, 2007, 47(2), 264-278.

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## Random ordering of compounds

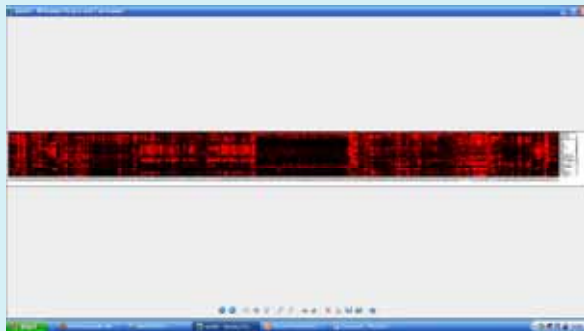


nACh receptor  
GABA A receptor  
Na K ATPase  
Voltage dependent Na channel  
Alpha-1 adrenergic receptor  
Dopamine receptor  
Acetylcholinesterase  
Monoamine oxidase  
iNOS expression  
NO in vivo  
cAMP dependent phosphodiesterase  
Calmodulin dependent protein kinase  
MLCK  
Protein kinase A  
Protein kinase C  
Topoisomerase II  
Apoptotic  
HIV-1 reverse transcriptase  
HIV-1 protease  
HIV-1 integrase  
Testosterone 5 alpha reductase  
Oestrogen receptor  
Aromatase  
Phospholipase  
Cyclooxygenase  
Lipoxygenase  
Adipose reductase

Herbal constituents

Targets

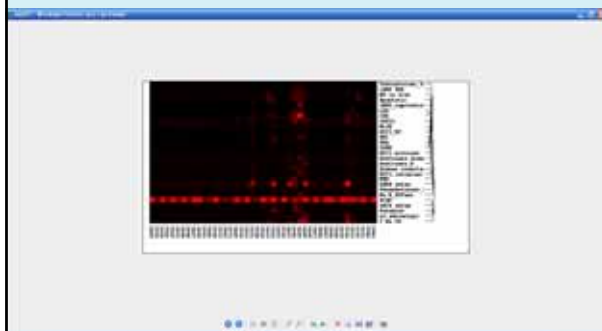
## Compounds ordered by chemical class



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## Compounds ordered by chemical class



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## Compounds ordered by chemical class



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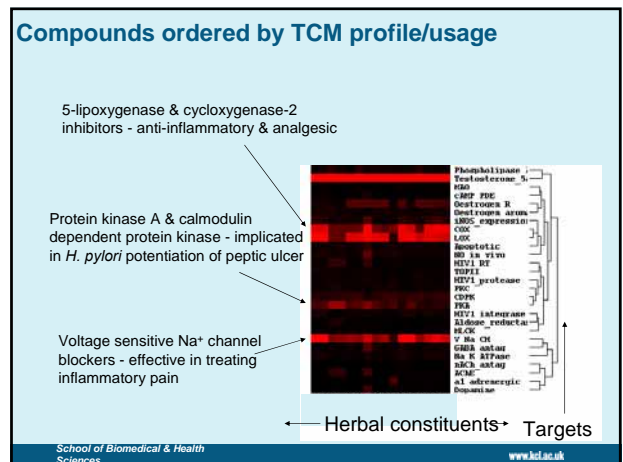
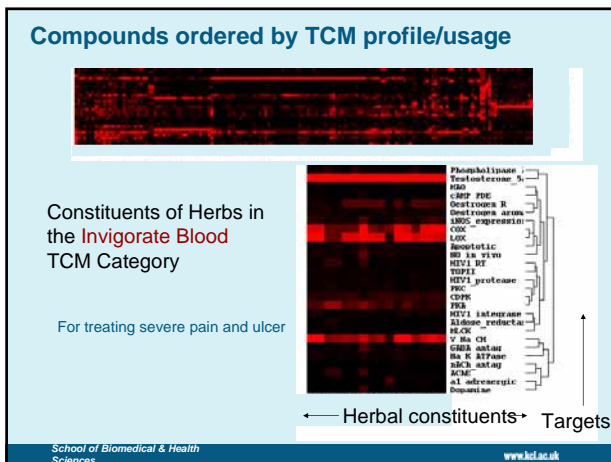
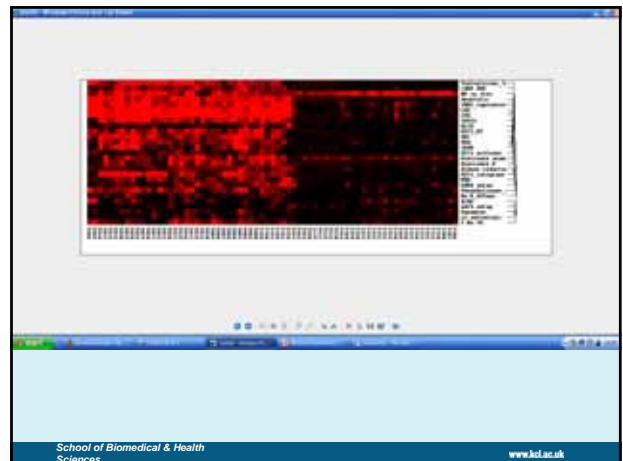
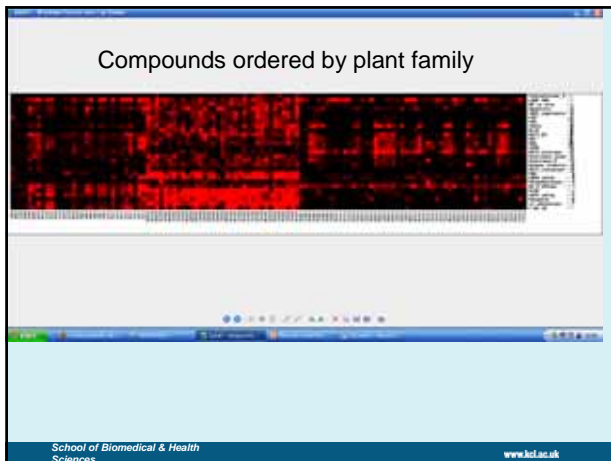
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## Compounds ordered by **plant family**



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## Application to database screening

Understand nature of drug-receptor interaction

Use mathematics to predict interactions (hydrophobic, van der Waal's, electrostatic and hydrogen bonding) between substrate and new molecules (not drugs) and calculate affinities

Obviously need precise 3D structures of compounds

Search for molecules with high binding affinity

Effectively screening of many thousands of compounds against target **without physically testing them**

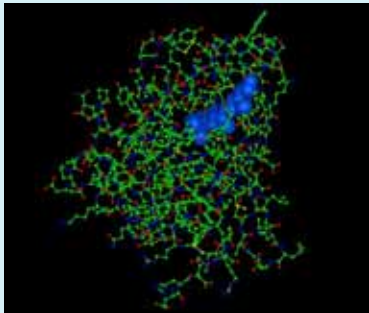
Screening *in silico*

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## Drug Discovery prediction verified by laboratory study

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## Dihydrofolate reductase with methotrexate at active site



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Perform correlation analyses of compound data against target, eg

- Receptor binding characteristics
- Enzyme inhibition

Using molecular modelling techniques

Hopefully find a class or classes over represented compared with chance

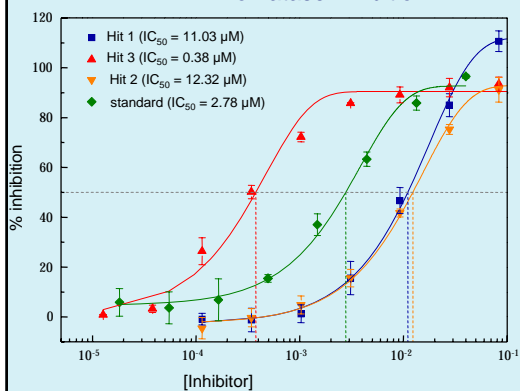
Screen those in detail

T M Ehrman, D J Barlow and P J Hylands, *Bioorganic and Medicinal Chemistry*, 2010, 18(6), 2204-2218.

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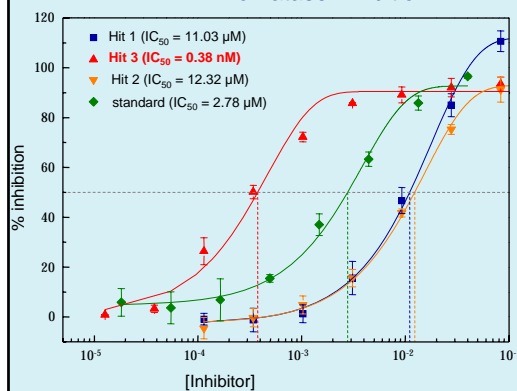
## Aromatase inhibition



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## Aromatase inhibition



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## The current situation

A few TCM-derived products in trial

One or two approved in the US

But these are principally fractions

- not really the whole medicine

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Summary

**We have improved tools now**

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We have better tools now

**We just have to do the work  
properly!!**

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**Thank you for your attention**

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