



**Good Practice in Traditional Chinese Medicine Research in
the Post-genomic Era**

GP-TCM

223154

D4.14

**Report of the discussion group on the use of functional
genomic techniques for *in vitro* CHM research**

Document description	
Name of document	Report of the discussion group on the use of functional genomic techniques for <i>in vitro</i> CHM research
Abstract	The present document includes D4.9 “ <i>Discussion Group evaluating data about molecular mechanisms of action</i> ” as explained in the related documentation. The discussion group originally intended to carry out the evaluation on a clinical theme proposed by WP6. The approach was later driven towards a more general approach centred on <i>in vitro</i> plant evaluation of molecular mechanisms, focusing on multiple mechanisms of action and systems biology. The WP4 coordination group therefore decided to merge the two deliverables into one. The issue of using functional genomic techniques for understanding molecular mechanisms in <i>in vitro</i> CHM research has been the main focus of the discussion group organized within WP4 members who answered to a specific questionnaire that was circulated. The answers were collected and analyzed and discussed during a meeting of WP4 coordination staff. The final document is thus organized into sub-paragraphs representing each question with a final conclusion.
Document identifier	D4.14
Document class	Deliverable
Version	1
Author(s)	Alessandro Buriani, Peter Hylands
Date of creation	30.03.2011
Date of last modification	29.04.2011
Status	Final
Destination	European Commission
WP number	WP4



TABLE OF CONTENTS

1 DISCUSSION GROUP ON USE OF FUNCTIONAL GENOMIC TECHNIQUES FOR <i>IN VITRO</i> CHM RESEARCH	5
1.1 Merging of D4.9 and D4.14	5
1.2 Introduction	5
1.3 Structure of the questionnaire	6
1.4 Results from the questionnaires and virtual discussion group on use of functional genomic techniques for <i>in vitro</i> CHM research	8
1.4.1 Question 1: What should be a correct approach to investigate molecular mechanism of action\molecular targets in CHM research?	8
1.4.2 Question 2: Can a Systems Biology approach overcome some of the problems hampering a full scale introduction of CHM <i>in vitro</i> research in the scientific mainstream?	10
1.4.3 Question 3: In TCM <i>in vitro</i> research rank the following technique in terms of usefulness?	11
1.4.4 Question 4: What are the main bottlenecks in this field?	14
1.4.5 Question 5: Can functional genomic techniques overcome basic problems in <i>in vitro</i> research on phytocomplexes, and how?	15
1.4.6 Question 6: To what extent are functional genomic techniques used among researchers in the CHM field?	16
1.4.7 Question 7: What are the main obstacles for the use of a functional genomics approach by researchers in the CHM field?	17
1.4.8 Question 8: How can <i>in-silico</i> tools be best applied to CHM research?	18
1.4.9 Question 9: Are <i>in silico</i> approaches readily available and what would be needed to potentiate their application in TCM research?	19
1.5 Conclusions	21
1.5.1 What should be a correct approach to investigate molecular mechanism of action\molecular targets in CHM research?	21
1.5.2 Can a Systems Biology approach overcome some of the problems hampering a full scale introduction of CHM <i>in vitro</i> research in the scientific mainstream?	22
1.5.3 In TCM <i>in vitro</i> research rank the following technique in terms of usefulness?	23
1.5.4 What are the main bottlenecks in this field?	25
1.5.5 Can functional genomic techniques overcome basic problems in <i>in vitro</i> research on phytocomplexes, and how?	26
1.5.6 To what extent are functional genomic techniques used among researchers in the CHM field?	27



1.5.7 What are the main obstacles for the use of a functional genomics approach by researchers in the CHM field?	27
1.5.8 How can in-silico tools be best applied to CHM research?	28
1.5.9 Are in silico approaches readily available and what would be needed to potentiate their application in TCM research?	29
Annex 1	31



1 DISCUSSION GROUP ON USE OF FUNCTIONAL GENOMIC TECHNIQUES FOR IN VITRO CHM RESEARCH

1.1 Merging of D4.9 and D4.14

The present document includes D4.9 (Discussion Group evaluating data about molecular mechanisms of action) as explained in the relative documentation. The discussion group originally intended to carry out the evaluation on a clinical theme proposed by WP6. The approach was later modified from an in-depth evaluation of the disease-related mechanisms, towards a more general problematic approach centred on *in vitro* plant evaluation of molecular mechanisms, including evaluation of multiple mechanisms of action and systems biology, in general terms without a therapeutic focus. This change has made D4.9 and D4.14 rather similar and hard to keep distinct. The WP4 coordination group therefore decided to merge the two deliverables into one.

1.2 Introduction

The identification of molecular mechanisms and targets represents critical step in the validation of a biological effect and - in the study of phytocomplexes as in Chinese Herbal Medicine research - this process is often hampered by the complexity of the molecular mixtures present in the drug, with many different molecules possibly contributing to the final effect, either in a positive or negative sense. With the advent of high throughput assays and – omics techniques it is now possible to examine simultaneous molecular effects which are likely to occur when using chemical mixtures and, with the help of bio-informatics, it is possible to look at such effects with a global view on the biological system affected. It is clear to everyone working in the field that this approach has a great potential, but many problems are limiting its application in the field of TCM, most of which are shared with the scientific community at large.

The issue of using functional genomic techniques for understanding molecular mechanisms in *in vitro* CHM research has been the main focus of the discussion group organized within WP4 members who were able to give their contribution to the issue with separate meetings and e-contacts, but the strategy chosen as a means to develop a consensus involved analysis of responses to a questionnaire prepared during a meeting in London in February 2011 by a working group at King's College and then further discussed via e-mail with other members. The questionnaire was circulated among WP4 members and collaborators, the answers collected and finally analyzed during a meeting of WP4 co-ordination staff in a second meeting in London, held late in March 2011.



1.3 Structure of the questionnaire

The text of the questionnaire circulated to WP4 members is presented below:

Introduction to the questionnaire

The following nine questions have been chosen by the WP4 coordination group, and will be used to collect WP4 members' different opinions on functional genomics techniques and in silico tools for in vitro CHM research. The answers to the questionnaire will be used as a collective interview aiming at establishing the state of the art of the use of functional genomics in CHM research with its pros and cons. The results of such a "virtual discussion group" will then be used to address deliverables WP4 D4.14 (Report of the discussion group on the use of functional genomic techniques for in vitro CHM research) and D4.9, which have been merged. In fact the discussion group on molecular mechanisms planned for D4.9 had to be updated after WP4 modified its approach to the issue, from an in-depth evaluation of the disease-related mechanisms towards a more general problematic approach centred on in vitro plant evaluation of molecular mechanisms. However this approach led to a change in the nature of the discussion, focusing not on disease areas but on approaches to the evaluation of multiple mechanisms of action and systems biology, without a therapeutic focus. This change made D4.9 complementary to D.4.14.

The present questionnaire is being circulated among WP4 members who are expected to return it to the WP4 coordination office. Here the answers will be collected and analyzed to address the deliverables.

Please answer each question using any approach you prefer, possibly with some explanation, where applicable.

The best results of this analysis will be achieved if each member will discuss each question with his/her colleagues in the workplace and give a collective answer to each point.

QUESTIONNAIRE ON THE USE OF FUNCTIONAL GENOMIC TECHNIQUES AND IN SILICO TOOLS FOR IN VITRO CHM RESEARCH

Name of WP4 member/s:

.....

Other non member participants (optional):

.....

QUESTION 1:

What should be a correct approach to investigate molecular mechanism of action\molecular targets in CHM research?

ANSWER 1:

QUESTION 2:

Can a Systems Biology approach overcome some of the problems hampering a full scale introduction of CHM *in vitro* research in the scientific mainstream?

ANSWER 2:

QUESTION 3:

In TCM *in vitro* research rank the following technique in terms of usefulness (explain the choice of the top ranking techniques)



Proteomics
Metabolomics
Genomics
Other –omic techniques
In silico models

ANSWER 3:

QUESTION 4:
What are the main bottlenecks in this field?

ANSWER 4:

QUESTION 5:
Can functional genomic techniques overcome basic problems in in vitro research on phytocomplexes, and how?

ANSWER 5:

QUESTION 6:
To what extent are functional genomic techniques used among researchers in the CHM field?

ANSWER 6:

QUESTION 7:
What are the main obstacles for the use of a functional genomics approach by researchers in the CHM field?

ANSWER 7:

QUESTION 8:
How can *in-silico* tools be best applied to CHM research?

ANSWER 8:

QUESTION 9:
Are *in silico* approaches readily available and what would be needed to potentiate their application in TCM research?

ANSWER 9:



1.4 Results from the questionnaires and virtual discussion group on use of functional genomic techniques for *in vitro* CHM research

The questionnaire was sent back from 10 out of 11 members and most of the questions were answered, few members did not feel they could answer questions 8 and 9, on *in silico* research, due to lack of expertise.

Note here too, that the latter questions were also used as key inputs for the scientific discussion developed during the *in silico* meeting in London (see D4.12)

1.4.1 QUESTION 1: What should be a correct approach to investigate molecular mechanism of action/molecular targets in CHM research?

Answers to question 1:

Member 1: In my opinion, it should be a mix of Molecular Biology Techniques. Before to consider the technique, one important question to address is the reproducibility of the studied systems, in terms of two important aspects: the use of a herbal mix, which components could vary from one to other preparations and the quality of each component of the mix in terms of contaminants or proportions of different compounds. As much as we controlled the herbal/single compounds to use, as better reproducibility of the results we will get. Synergism or antagonism between herbal compounds should be taken into account.

Gene expression analysis are appropriated (gene signature) but higher levels of gene expression control such as miRNAs should not be forgetting. Posttranslational protein modification analysis could be also pertinent. Finally, functional studies *in vivo* and *in vitro* modulating the molecular targets identified should be performed as a final proof of correct target identification..

Member 2: I think it is not possible to define the exact molecular mechanism or to identify the ultimate target(s) of compound mixtures. At best we can describe multiple effects that are mediated by this mixture (which is indeed sometimes “sold” as molecular mechanism).

Member 3: A combination of classical biochemical signaling work and combination with “state of the art” target fishing procedure, live cell imaging, and –omics technology

Member 4: A combination of conventional molecular biology and other advanced technique

Member 5: Ideally, a combination of conventional molecular biology methods, “omics” technology, bioinformatics, systems biology and other emerging technologies.

Member 6: A correct procedure should consist in a well-consolidated pipeline composed of different approaches:

- *In silico* evaluation of putative ligands on different targets



- In vitro validation of the positive hits through cellular molecular pharmacology assays
- In vivo validation of wet results on animal models for the investigated pathologies

Member 7: To achieve reliable identification of the molecular targets and the active compounds/mixtures of compounds that mediate the CHM action, very important and often underestimated factor is thorough quality control of the starting material. Furthermore, it seems reasonable to well keep in mind the possibility that the final outcome could be due to a synergistic or additive action of different chemical constituents present in the crude herbal drug. Before trying to identify the molecular targets mediating the action of the CHM, the most straight-forward approach would be first to identify single compounds, or mixtures of well defined single compounds that would have similar effectiveness as the complex crude drug in respect to a reliable functional readout. Having single compounds or well defined mixtures with known composition would assure reliability and reproducibility of the findings, and next step than would be to attempt to identify the single molecular target(s) that account for the observed functional effect.

Member 8: A deterministic approach to investigate molecular mechanisms of action or molecular targets is nowadays showing its limits, and there is a growing consensus in the scientific community that focusing into single molecular contexts as if they were isolated entities has a very limited value for a correct approach to pharmaco-toxicological studies. This is particularly apparent when dealing with research on herbal drugs, where multiple active components are present. Today a Systems Biology approach allows us to get closer to a real view of the multiple and simultaneous effects exerted by phytocomplexes on biological systems. This is true also when using single active components; in fact the complexity issue is still true when considering single molecules, which directly or indirectly can affect multiple biological targets.

Using functional genomics techniques can allow to do initial screening directly on the molecular targets affected, for instance using DNA microarrays. Once identified, clusters of interrelated molecular targets can be investigated more in deep, using –omics techniques like proteomics or metabolomics. Basically, while previously the focus on the mechanism would be led by the observed biological effect, now, using high throughput analysis it is possible to reach directly that information in the very beginning of a research project.

Member 9: (a) In silico followed by in vitro approach. Computational models (docking, ligand-based, pharmacophores) first used to screen phytochemical libraries, followed by in vitro confirmation. (b) Molecular modeling used to investigate likely mode of action (eg. docking) of in vitro results. For genomic-scale screening, approach (a) is the most feasible.

Member 10: Any molecular biology method, conventional or advanced technique, it depends on the question you want to give an answer



1.4.2 QUESTION 2: Can a Systems Biology approach overcome some of the problems hampering a full scale introduction of CHM *in vitro* research in the scientific mainstream?

Answers to question 2:

Member 1: In my opinion, apart from the fact that molecular techniques could reveal TCM molecular mechanisms of action, Systems Biology approaches could be a powerful tool for herbal mixture quality control and standardization of TCM treatments, that, in my personal opinion is one of the main walls to be knocked down for TCM research.

Member 2: Once a considerable amount of really meaningful data are published the acceptance in the scientific community will probably rise. Though I would not expect to become CHM scientific mainstream.

Member 3: Yes of course, however the bioinformatic analysis is of major importance

Member 4: May be, but more evidence is needed

Member 5: Possible, but more evidence is clearly needed.

Member 6: In my opinion, systems biology is not useful for overcoming specific problems. It should be implemented in the final stages of the research for networking all the data collected during the previous steps.

Member 7: The complexity of CHM is a very big challenge, which makes it very difficult to track the main molecular targets and mechanisms mediating CHM action. Systems Biology approaches have the advantage that with them the researchers get access to the “full-scale picture” of all changes that are happening after CHM treatment, which might give valuable clues about the main affected signaling events and pathways. Knowledge for the main affected signaling pathways would then greatly facilitate a more focused research effort aiming to reveal from one side the single active compounds of the CHM, and from other side the single affected molecular targets within the affected pathway.

Member 8: Systems Biology zooms out the focus from the detail to the system in its dynamic complexity. This approach allows an analysis of the mechanisms of action of active molecules on direct and indirect targets down to the changes of the biological homeostatic equilibrium. This is particularly important when testing phytocomplexes and especially formulations, like in CHM, given that each component can have multiple targets linked to the pharmacological and toxicological activity. A systems biology approach thus seems



particularly fit for studying CHM, providing a strategy for collecting every detail of each system to merge them together in a complex or holistic vision. Today with the same principle a novel medical approach is emerging, personalized Medicine, where the subject is seen as a whole with all its molecular characteristics which distinguish it from the rest. This new vision, affecting both biology and medicine, is actually providing a special ground to overcome one of the main cultural problems for the diffusion of TCM in the western scientific cultural mainstream: a holistic vision versus a deterministic one. Indeed this contradiction has no reason to exist any longer in the era of Systems Biology, and this will give a fundamental contribution to the full scale introduction of CHM in the scientific mainstream.

Member 9: Yes. For example, in the case of signal transduction or metabolic pathways, network analysis may help to simplify the range of targets to be considered, in the first instance, by identifying critical components (eg. those showing high 'centrality').

On data sets containing large numbers of in silico predictions against a large battery of targets, then clustering of target fingerprints should also prove informative.

1.4.3 QUESTION 3: In TCM in vitro research rank the following technique in terms of usefulness (explain the choice of the top ranking techniques)

- Proteomics
- Metabolomics
- Genomics
- Other –omic techniques
- In silico models

Answers to question 3:

Member 1: Depends what you want to study. For quality control and setting up of robust in vitro and in vivo models of study, genomics (very useful for in vitro studies), proteomics and metabolomics (necessary for in vivo models). For further analyses of molecular mechanisms of action, we should also applied in silico models and other omic and non-omic techniques. 1.

Member 2: from Genomics to Proteomics it is getting more complex. Thus, the difficulties of an overall meaningful interpretation will probably rise from Genomics to Proteomics. On the other hand, these techniques should be even combined to get a meaningful picture of CHM effects. Without professional bio-informatics the outcome would be most likely limited.

Metabolomics/metabolic profiling (of plant constituents) in combination with functional assays may help in the field of quality control.

In silico tools can be extremely useful to find a direction where to go, but cannot be used a stand-alone technique.

Member 3:

- Proteomics 1



- Metabolomics 2
- In silico models 3
- Genomics 4
- Other –omic techniques 5

Member 4: It depends on what you want to demonstrated. If the transcriptional regulation is the main hypothesis, genomics have been shown sensitive and specific; in the case you would study biological processes involving proteins, proteomic should be used; if you would study an effect on metabolism, metabolomics is the first choice

Member 5: It depends on your hypothesis and the individual applications. For a hypothesis focusing on transcriptional regulation, genomic/transcriptomic tools have been shown to be both sensitive, specific and affordable; for biological processes in which proteins and peptides are of the main concern, proteomics should be used, although it might not be as sensitive and specific as microarray yet; finally, if the metabolic profile is the main concern, metabolomics is the first choice. Ideally, a combination of multiple technologies including omics, conventional molecular biology technology, activity and/or functional assays and imaging tools could be most powerful.

Member 6: No usefulness ranking should be done. All these techniques should be implemented according to a specific time of the research:

1. In silico models
2. Proteomics
3. Metabolomics
4. Genomics

Member 7:

1. Genomics (most useful);
2. Proteomics;
3. Metabolomics;
4. In silico models;
5. Other –omic techniques.

The readout obtained with Genomics is less complicated and therefore might represent a better alternative than Proteomics and Metabolomics. Genomics is just taking account for differences in gene expression, whereas Proteomics readout is influenced on further events such as alternative splicing, rate of translation, posttranslational modifications etc which makes it more complicated. Metabolomics has also a further degree of complexity, because it is not influenced just from gene expression and from protein expression, but also from external influences like for example the dietary intake of the respective metabolite(s). To investigate such a complex drugs such as CHM, it might represent a good choice to start with



System biology approach that is possibly simpler and more upstream (in that case Genomics). In silico models are rated lower than the three –omics approaches, since most often now-days the in silico approaches are still having too low predictive power, and the value of the real experimental data is still much bigger.

Member 8:

- 1 - Proteomics
- 1 – Metabolomics
Proteomics and metabolomics, allow a precise and complex observation of the downstream molecular effects, both direct and indirect, some of which might be more relevant or evident on proteins and some others on metabolites, depending on the kind of global biological effect. So the two –omics can be considered equally relevant and might be applied more conveniently to different experimental contexts.
- 2 - In silico models. In silico models allow an efficient and rapid interpretation of experimental data to read their meaning in complex systems, which otherwise in many cases would not be possible. They can be essential to obtain a unique (holistic) vision of many different molecular effects. They can also be used for simulations which can speed up in vitro research.
- 3 – Genomics. DNA arrays can be used as a screening tool before proceeding towards techniques more focused on downstream effects. Another experimental use of genomics is herbal DNA fingerprinting which has a very high value in standardization of plant material.
- 4 - Other –omic techniques: though valuable, these techniques are still poorly used, so more work is needed to reach a more standard and shared view of these –omics in the scientific community

Member 9: I would rank metabolomic and in silico approaches at the top. Metabolomics offers the most appealing approach both to identifying cellular responses to a particular CHM compound, and to understanding how that response is modified in the case of additional compounds (ie. one of the most revealing ways to study synergy). In silico approaches provide a 'theoretical' arm to the study of CHM, without which a unifying framework is that much more difficult.

Member 10: It depends on what you want to demonstrate: if it is an effect on metabolism: metabolomics, an effect at gene level genomics etc. In case you would correlate gene transcription and protein levels, both transcriptomic and metabolomics.



1.4.4 QUESTION 4: What are the main bottlenecks in this field?

Answers to question 4:

Member 1: The quality control of the herbs/mixtures/single compounds we will applied in experimental models

Member 2: Data evaluation and interpretation → the production of really conclusive meaningful results; collaborations with bio-informatics needed

Member 3: Complexity and its meaningful interpretation

Member 4: The variability of the starting material

Member 5: Although all “omics” technologies have been more sensitive, specific, powerful and affordable then ever before, the current funding to TCM and other herbal medicine research in Europe is lacking, making it the top bottleneck to bring these powerful technologies in research of complex preparations such as Chinese herbal medicines.

Member 6: Several of the targets of drugs/active molecules are proteins. The identification of potential targets involved in specific pathologies, and the knowledge of their structures are difficult to obtain but necessary for all the subsequent steps.

Member 7: The variability of the quality of the starting material, which impairs the reproducibility of the obtained scientific results.

Member 8: though knowledge is fast growing in the field, its translation to student education programs is still not satisfactory. This education gap is rather tangible among research scientists and this slows down their approach to functional genomics application. Moreover, in the context of in vitro research, scientists have been using experimental models that do not allow functional genomics to express their full potential. An example is given by the use of single molecules or single cells, while functional genomics would allow to look at entire biological systems. In CHM research where in many cases it would be possible to use human samples like blood or urines, this is particular relevant, since it would permit to be in close contact with the clinical use.

Many western scientists do not even get close to CHM research, also because is not officially used in many western countries and this gives it a low political priority at a single state level for financing purposes.

The other point which is also a direct consequence of the others is that few herbal research centers do actually have the possibility of conducting research using a functional genomics approach. The techniques require instruments and expertise which are hardly available for in vitro research.



Member 9: Many. (a) lack of information and appropriate databases (though much improved compared to several years ago); (b) lack of personnel with appropriate skills; (c) lack of funds (?). Another point – to what extent are efforts being made to integrate TCM with Western biomedicine? Are we in danger of taking the ‘traditional’ out of TCM?

Member 10: Fund availability, and the transfer of *in vitro* results to *in vivo* situation: see my reply to question 5

1.4.5 QUESTION 5: Can functional genomic techniques overcome basic problems in *in vitro* research on phytocomplexes, and how?

Answers to question 5:

Member 1: As I mentioned before, these techniques could help a lot in the control of reproducibility of the systems, among to shed light into mechanisms involved in TCM *in vitro* effects.

Member 2: At least they may complement our tools and help in addition with more conventional cell and molecular biological techniques to get a clearer picture.

Member 3: No. I do not think so

Member 4: All omics and conventional methodologies could help for to study both toxic and therapeutic profile of TCM materials.

Member 5: A combination of omics and conventional methodologies should find its use in controlling the quality of research materials, suggesting both toxic and therapeutic effects, as well as defining mechanisms of actions and signaling pathways, etc.

Member 7: As already outlined in Answer 2, functional genomic techniques have the advantage that with them the researchers get access to the “full-scale picture” of all changes that are happening after CHM treatment, which might give valuable clues about the main affected signaling events and pathways. Based on the outcome of the functional genomic techniques the researchers could focus stronger their efforts in investigation of selected signaling pathways that are affected from the investigated CHMs.

Member 8: Some of the basic problems linked to the *in vitro* use of phytocomplexes are due to the limits of experimental models themselves, where only a biological effect or one of its molecular aspects are considered. A more global vision of the biological systems in their complexity and interconnections can help to overcome such experimental limit. The use of a



functional genomics approach, which allows to look at many different molecular targets within the same experimental complex, needs to be driven towards a view of the whole and not the simple sum of many different molecular variations. In this case using a phytocomplex might not be so different from using a single molecule. One important issue which cannot be addressed by the use of functional genomics is linked to the variability of the phytocomplex itself, leading to poor standardization of the experimental samples, even though, thanks to DNA fingerprinting techniques and metabolomic characterization of plant material is now possible to obtain reference standards. This can certainly help, but still does not address the issue of reducing sample variability.

Member 9: I'm not an expert on many aspects of functional genomics, but I think the answer must be 'yes', as in what I said about metabolomics and herbal synergies (*in silico* 'chemogenomics' is, I think, equally valid in this respect, though approaching it from a different angle).

Member 10: It helps but the results may be misleading if it is not known how the phytocomplex is absorbed and metabolized etc *in vivo*. In other words: what you test in a well, is not what you find systemically.

1.4.6 QUESTION 6: To what extent are functional genomic techniques used among researchers in the CHM field?

Answers to question 6:

Member 1: Up to my knowledge, they are not much papers showing genomic analysis in TCM. It seems that more articles are published applying functional genomics studies *in vitro* than *in vivo*. In any case, not many of them are reliable since quality control of the herbs and reproducibility of the systems used for performing studies are guaranteed.

Member 2: Currently the use is, as far as I can judge this, limited to a few groups.

Member 3: To a high extent, however I am not convinced that it will add considerable Robust knowledge

Member 4: The application functional genomic techniques is an area of big promise in CHM research.

Member 5: As far as I know, not many. For example, to learn how many papers have studied CHM using "omics" and microarray technology, we searched Pubmed using the following searching strategies and only fewer than 166 papers were found (as of 25th March 2011): (gene array [mesh] OR genomics [mesh] OR DNA microarray [mesh] OR Oligonucleotide



Array Sequence Analysis [mesh] OR metabolomics [mesh] OR proteomics [mesh] AND (Drugs, Chinese Herbal [mesh] OR Medicine, Chinese Traditional [mesh] OR Medicine, Oriental Traditional [mesh])

Member 7: The application functional genomic techniques in CHM is still an area of research, which however is of big promise.

Member 8: despite their potential, their use is very limited.

Member 9: Not a lot at present as far as I'm aware.

Member 10: I would need to make a pubmed search, in order to reply. I suppose not much used

1.4.7 QUESTION 7: What are the main obstacles for the use of a functional genomics approach by researchers in the CHM field?

Answers to question 7:

Member 1: Technically, I do not see big problem, since most of the TCM scientist could find experts or platforms to be help with the genomic performance, even companies can do it. But, once again, quality control should be assured.

Member 2: Data evaluation and interpretation → the production of really conclusive meaningful results; collaborations with bio-informatics needed

Member 3: As I said the high extent of complexity which ask for "prioritisation"

Member 4: Probably funding

Member 5: Funding is the key issue. In addition, accessibility to core omics facilities and related bioinformatics services, the poor knowledge about the affordability, promises and pitfalls of using these technologies and the fears of the huge amount of information these technologies could generate also switch researchers off.

Member 7: To successfully integrate the complexity of CHM with functional genomics approaches which are also very complex and often yield results that are difficult to interpret, it needs a strong expertise and joint work of scientists from many different fields of research: experts in TCM, phytochemists, experts in molecular biology/pharmacology/medicine, and often even informatics (since the evaluation and interpretation of the huge amount of data generated with the functional genomics approaches often is not very straight-forward).



Member 8: lack of expertise in functional genomics by many herbal *in vitro* researchers – difficulties in finding equipment available for *in vitro* research –in general, public funding is rather limited in western countries for traditional medicine studies, projects using functional genomics instead require high costs especially in the start up phase, where new updated technology is needed.

Member 9: I can only really answer for *in silico* chemogenomics. Here the main obstacles are patchy data (herbal compounds, biological activities (eg. for constructing or validating predictive models), relevant PDB entries etc.). There is also the question of appropriate software for high throughput screening, reliability of the approaches on which it is based, and the question of whether single models are really sufficient, in the case of *in silico* prediction, to justify basing *in vitro* studies around them.

Member 10: Probably funding, but also low expertise and knowledge in general

1.4.8 QUESTION 8: How can *in-silico* tools be best applied to CHM research?

Answers to question 8:

Member 1: In my opinion, they can be used as predictive tools for pointing out or directing molecular targets or mechanisms involved in TCM actions. They might narrow the amount of targets to be checked. Additionally, they are useful for interpretation of omics results.

Member 2: Next to the mandatory use of bio-informatics in data evaluation/interpretation, *in silico* tools such as pharmacophore modeling and *in silico* screening may be used to get first ideas about biological targets (if there is any knowledge about the chemical structure of single compounds available)

Member 5: Built on ever–strengthening bioinformatics technologies, *in silico* tools could be useful in finding patterns, pathways, effector/mediator clusters and help forge new hypothesis and guide future research.

Member 6: The *in silico* tools are very useful in the first steps of pharmacological research. Computational approaches allow us to evaluate a lot of molecules on several targets with a minimal economic effort. The positive hits can subsequently be investigated through *in vitro* and/or *in vivo* approaches, which are more expensive and associated to a lower throughput power.



Member 7: For evaluation and interpretation of the results obtained from functional genomics approaches.

Member 8: *in silico* tools and more in general bioinformatics/computer biology are just essential for functional genomics application, both for multiple data analysis and systems modelling. Without their analytical power would be impossible to approach such complex experimental tools.

Member 9: Chemogenomics may be the single most important objective. However, even in the event that many targets of many compounds can be predicted with reasonable accuracy, there remains the question of how best to use that data. This requires input from bioinformatics (gene/disease relationships), and also TCM. Bridging the gulf between medical paradigms is something to which data mining is well suited.

1.4.9 QUESTION 9: Are *in silico* approaches readily available and what would be needed to potentiate their application in TCM research?

Answers to question 9:

Member 1: Today, *in silico* approaches are mostly available for most of the scientist, although good use and successful results from their use required a strong knowledge of them. On the other hand, to get results from *in silico* approaches, the scientifically question to be solved should be accurately formulated to avoid getting tons of non-usable or non-interpretable information. Finally, *in silico* results must be confirmed by other molecular and cellular approaches.

Member 2: Here strong collaborations are needed. And collaborations are only readily available when the scientific field attracts expert in this field.

Member 5: I think *in silico* approach is been used all the time in such an Information Technology era. For example, analysis of conventional and “omics” data are routinely carried out using computerised tools worldwide. There are also many databases with specialised analysing powers, which are developed in the past years, but they are usually poorly maintained, updated and their accessibility is generally poor. Meanwhile there is much duplication in efforts, re-spinning the wheels and wasting limited resources.

Sustainable support from governments, charities and the industrial community is needed for establishing authoritative and well-maintained systems; reliable multiple language translation is another on our wish list; open access or easy access will also make a great difference.



Member 6: The use/implementation of *in silico* approaches require specific expertises in computer science and computational chemistry, since the risk of producing biased results is not null. An efficient application of *in silico* approaches requires the chemical characterization of putative active compounds and their organization in chemical/structural databases.

Member 7: *In silico* approaches and tools that facilitate and complement molecular biology studies are very dynamic research area that quickly develops. Direct interaction with experts from that area is still is the best option to maximise the value of the obtained *in silico* predictions.

Member 8: what is really needed is a better crosstalk between the *in vitro* and the *in silico* experts as well as an improved academic curriculum for *in vitro* scientists in order for them to better understand the language and the potentialities of computer science for their research

Member 9: Most of the tools that have been developed so far either revolve around data (generally fairly simple inventories of relevant compounds) and docking (eg. TarFisDock and iScreening). iScreening will apparently dock 20,000 compounds from CHM against any PDB receptor which the user uploads, and send you a list of the best 200 compounds.

The main difficulties are that many of the databases are difficult to use or access. The efficacy of the current docking tools is also questionable (in terms of speed, reliability etc.). Though they are, for the most part, easy to operate, they are not suitable for those with no background in virtual screening, and the results are intended for individual use (though there is probably no reason why they can't be collected by the provider in the form of a database). However, as yet, here is no centralized repository which combines information both on compounds and targets, traditional uses and so on, which can be easily accessed by users from a wide variety of backgrounds.

So far, it is therefore the case that *in silico* approaches are at a fairly low level of organization/sophistication. Raw data and docking results for a limited number of compounds and/or targets comprise the first stage. There are further layers of analysis that need to be undertaken before a more complete picture begins to emerge. As mentioned above, these include bioinformatics and TCM.

Many of the appropriate tools are now in place, though refinements to most of them will continue to be made. That may not be as important as the application of different methods of virtual screening to compound databases (e.g. ligand-based, structure-based pharmacophores and docking), and the use of results based on consensus (which should become progressively easier).



1.5 Conclusions

1.5.1 What should be a correct approach to investigate molecular mechanism of action\molecular targets in CHM research?

Most members agree on the fact that one single methodology is not sufficient to investigate a molecular mechanism of action in CHM: M1 “a mix of *Molecular Biology Techniques*”; M3 “A combination of *classical biochemical signaling work and combination with “state of the art” target fishing procedure, live cell imaging, and –omics technology*”; M4 “A combination of *conventional molecular biology and other advanced technique*”; M5 “a combination of *conventional molecular biology methods, “omics” technology, bioinformatics, systems biology and other emerging technologies*”; M6 “a well-consolidated pipeline composed of different approaches, *in silico evaluation of putative ligands...in vitro validation...through cellular molecular pharmacology...in vivo validation...*”; M8 “*functional genomics techniques can allow to do initial screening directly on the molecular targets affected, for instance using DNA microarrays. Once identified, clusters of interrelated molecular targets can be investigated more in deep, using –omics techniques like proteomics or metabolomics.*”; M9 “*In silico followed by in vitro approach. Computational models (docking, ligand-based, pharmacophores) first used to screen phytochemical libraries, followed by in vitro confirmation. Molecular modeling used to investigate likely mode of action (eg. docking) of in vitro results*”; M10 “*Any molecular biology method... depends on the question*”.

Some indications are also given on the experimental design: M7 “*first to identify single compounds, or mixtures of well defined single compounds that would have similar effectiveness as the complex crude drug in respect to a reliable functional readout. Having single compounds or well defined mixtures with known composition would assure reliability and reproducibility of the findings, and next step than would be to attempt to identify the single molecular target(s) that account for the observed functional effect*”; M11 “*Gene expression analysis (gene signature) but higher levels of gene expression control such as miRNAs should not be forgetting. Posttranslational protein modification analysis could be also pertinent. Finally, functional studies in vivo and in vitro modulating the molecular targets identified should be performed to validate the molecular findings*”;

The main differences emerging among members in this case are strictly related to the experimental approach, data-driven (bottom-up), model-driven (top-down) or question-driven (middle-out), which of course can change the types of methodologies that can be used and their importance.

One member points out some differences brought up by the novel systems biology approach in the experimental work carried out with herbal preparations like in CHM: M8 “*A deterministic approach to investigate molecular mechanism of action or molecular targets is nowadays*



showing its limits, and focusing into single molecular contexts as if they were isolated entities has a very limited value for a correct approach to pharmaco-toxicological studies. This is particularly apparent when dealing with research on herbal drugs, where multiple active components are present. Today a Systems Biology approach allows us to get closer to a real view of the multiple and simultaneous effects exerted by phytocomplexes on biological systems"

One important issue raised is the variability and the quality of the experimental sample material, which can drastically affect reproducibility and thus should be addressed even before starting any experimental project with CHM: M1 "*Before considering the technique, one important question to address is the reproducibility, in terms of two important aspects: variability and quality control of the herbal mix. Synergism or antagonism between herbal compounds should also be taken into account.*"; M7 "*very important and often underestimated factor is thorough quality control of the starting material. Furthermore, it seems reasonable to well keep in mind the possibility that the final outcome could be due to a synergistic or additive action*"

One member has an opinion apparently different from the rest, questioning even the possibility of identifying molecular mechanisms when using herbal preparations: M2 "*it is only possible to describe multiple effects that are mediated by mixtures of compounds (sometimes "sold" as molecular mechanism).*"

1.5.2 Can a Systems Biology approach overcome some of the problems hampering a full scale introduction of CHM in vitro research in the scientific mainstream?

Several members think that systems biology can contribute to the full scale introduction of CHM in the scientific mainstream, thanks to its wider view of the biological systems, essential for investigating mixtures of compounds: M3 "*Yes of course, however the bioinformatic analysis is of major importance*"; M7 "*Systems Biology approaches have the advantage that with them the researchers get access to the "full-scale picture" of all changes that are happening after CHM treatment.....Knowledge for the main affected signaling pathways would then greatly facilitate a more focused research effort aiming to reveal from one side the single active compounds of the CHM, and from other side the single affected molecular targets within the affected pathway.*"; M9 "*...network analysis may help to simplify the range of targets to be considered, in the first instance, by identifying critical components...On data sets containing large numbers of in silico predictions against a large battery of targets, then clustering of target fingerprints should also prove informative.*"; one member also highlights the holistic view of systems biology, and how it can contribute to the acceptance in the scientific community of TCM: M8 "*Systems biology zooms out the focus from the detail to the system in its dynamic complexity. This approach allows an analysis of the mechanisms of action of active molecules on direct and indirect targets.....This is particularly important when testing phytocomplexes, like in CHM, given that each component can have multiple targets linked to*



the pharmacological and toxicological activity. A systems biology approach thus seems particularly fit for studying CHM, providing a strategy for collecting every detail of each system to merge them together in a complex or holistic vision.....This new vision, affecting both biology and medicine, is actually providing a special ground to overcome one of the main cultural problems for the diffusion of TCM in the western scientific cultural mainstream: a holistic vision versus a deterministic one. Indeed this contradiction has no reason to exist any longer in the era of Systems Biology, and this will give a fundamental contribution to the full scale introduction of CHM in the scientific mainstream"; One member also thinks that systems biology can solve other problems related to the variability of herbal preparations and quality control: M1 *"system biology approaches could be a powerful tool for herbal mixture quality control and standardization of TCM treatments, that, in my personal opinion is on of the main walls to be knocked down for TCM research";*

Three members think that the time is not ripe yet for a definite answer to the question: M2 *"Once a considerable amount of really meaningful data are published the acceptance in the scientific community will probably rise";* M4 *"May be, but more evidence is needed";* M5 *"Possible, but more evidence is clearly needed".*

One member does not think that systems biology will allow researchers to solve specific problems; M6 *"...is not useful for overcoming specific problems. It should be implemented in the final stages of the research for networking all the data collected during the previous steps".*

1.5.3 In TCM *in vitro* research rank the following technique in terms of usefulness (explain the choice of the top ranking techniques)

Proteomics
Metabolomics
Genomics
Other –omic techniques
In silico models

Most members think that it really depends on the nature of the experimental question with the methodology chosen as most appropriate being dependent on the intended scope of the study: M1 *"Depends what you want to study. For quality control and setting up models of study, genomics (very useful for in vitro studies), proteomics and metabolomics (necessary for in vivo models). For further analyses of molecular mechanisms of action, we should also applied in silico models and other omic and non-omic techniques";* M4 *"It depends on what you want to demonstrate. If the transcriptional regulation is the main hypothesis, genomics have been shown sensitive and specific; in the case you would study biological processes involving proteins, proteomic should be used; if you would study an effect on metabolism, metabolomics is the first choice";* M5 *"It depends on your hypothesis and the individual applications. For transcriptional regulation, genomic/transcriptomic tools have been shown to be both sensitive, specific and affordable; for biological processes in which proteins and*

peptides are of the main concern, proteomics should be used, although it might not be as sensitive and specific as microarray yet; finally, if the metabolic profile is the main concern, metabolomics is the first choice.”; M6 “No usefulness ranking should be done. All these techniques should be implemented according to a specific time of the research: 1. In silico models 2. Proteomics 3. Metabolomics 4. Genomics”; M10 “It depends on what you want to demonstrate: if it is an effect on metabolism: metabolomics, an effect at gene level genomics etc.”

Some also advocate the combined use of methods: M5 “Ideally, a combination of multiple technologies including omics, conventional molecular biology technology, activity and/or functional assays and imaging tools could be most powerful.”; M10 “In case you would correlate gene transcription and protein levels, both transcriptomics and proteomics”;

For those members who did give a rank, there is no consensus, apart from the ranking of other –omic techniques, which is at the bottom, while: M3 “Proteomics 1, Metabolomics 2, In silico models 3, Genomics 4, Other –omic techniques 5”; M7 “1 Genomics (most useful); 2. Proteomics; 3. Metabolomics; 4. In silico models; 5. Other –omic techniques”; M8 “1. Proteomics; 1. Metabolomics; 3. In silico models; 4 Genomics; 5. Other –omic techniques”; M9 “Metabolomic 1, in silico 2”. There were also reasons given by some: M9 “...Metabolomics offers the most appealing approach both to identifying cellular responses to a particular CHM compound, and to understanding how that response is modified in the case of additional compounds.... In silico approaches provide a ‘theoretical’ arm to the study of CHM, without which a unifying framework is that much more difficult.” M8 “Proteomics and metabolomics, allow a precise and complex observation of downstream molecular effects, both direct and indirect. In silico models allow an efficient and rapid interpretation of experimental data in complex systems, which otherwise in many cases would not be possible. They can be essential to obtain a unique (holistic) vision of many different molecular effects. They can also be used for simulations which can speed up in vitro research. DNA arrays can be used as a screening tool before proceeding towards techniques more focused on downstream effects. Another experimental use of genomics is herbal DNA fingerprinting which has a very high value in standardization of plant material”; The complexity in using the methodology is also an issue affecting their usability: M2 “from Genomics to Proteomics it is getting more complex. Thus, the difficulties of an overall meaningful interpretation will probably rise from Genomics to Proteomics. On the other hand, these techniques should be even combined to get a meaningful picture of CHM effects. Without professional bio-informatics the outcome would be most likely limited. Metabolomics/metabolic profiling (of plant constituents) in combination with functional assays may help in the field of quality control. In silico tools can be extremely useful to find a direction where to go, but cannot be used a stand-alone technique”; M7 “The readout obtained with Genomics is less complicated and therefore might represent a better alternative than Proteomics and Metabolomics. Genomics is just taking account for differences in gene expression, whereas Proteomics readout is influenced on



further events such as alternative splicing, rate of translation, posttranslational modifications etc. Metabolomics has also a further degree of complexity, because it is not influenced just from gene expression and from protein expression, but also from external influences like for example the dietary intake of the respective metabolite(s). To investigate complex drugs such as CHM, it might represent a good choice to start with System iology approach that is possibly simpler and more upstream (in that case Genomics). In silico models are rated lower than the three –omics approaches, since most often now-days the in silico approaches are still having too low predictive power, and the value of the real experimental data is still much bigger”

1.5.4 What are the main bottlenecks in this field?

Many members indicate the poor funding policies as the main problem for the wider use of functional genomics for CHM: M7 *“the current funding to TCM and other herbal medicine research in Europe is lacking, making it the top bottleneck to bring these powerful technologies in research of complex preparations such as Chinese herbal medicines”*; M5 *“Although all “omics” technologies have been more sensitive, specific, powerful and affordable than ever before, the current funding to TCM and other herbal medicine research in Europe is lacking, making it the top bottleneck to bring these powerful technologies in research of complex preparations such as Chinese herbal medicines”*, M8 *“Many western scientists do not even get close to CHM research, also because is not officially used in many western countries and this gives it a low political priority at a single state level for financing purposes”*; M9 *“lack of funds”*; M10 *“Fund availability”*. The second most important bottleneck is considered sample variability and quality control: M1 *“quality control of the herbs/mixtures/single compounds”*; M4 *“The variability of the starting material”*; M7 *“variability of the quality of the starting material, which impairs the reproducibility”*.

The complexity in data evaluation and interpretation, which require specific and different expertise, an issue which should be addressed starting from students’ curricula: M2 *“Data evaluation and interpretation → the production of really conclusive meaningful results; collaborations with bio-informatics needed”*; M3 *“Complexity and its meaningful interpretation”*; M6 *“identification of potential targets involved in specific pathologies, and the knowledge of their structures are difficult to obtain”*; M8 *“though knowledge is fast growing in the field, its translation to student education programs is still not satisfactory. This education gap is rather tangible among research scientists and this slows down their approach to functional genomics application. Moreover, in the context of in vitro research, scientists have been using experimental models that do not allow functional genomics to express their full potential. An example is given by the use of single molecules or single cells, while functional genomics would allow to look at entire biological systems. In CHM research where in many cases it would be possible to use human samples like blood or urines, this is particular relevant, since*

it would permit to be in close contact with the clinical use.”; and also” few herbal research centers do actually have the possibility of conducting research using a functional genomics approach. The techniques require instruments and expertise which are hardly available for in vitro research. “ M9 ”lack of personnel with appropriate skills”.

One member, M9, also points out the “*lack of information and appropriate databases*” and, finally asks “*to what extent are efforts being made to integrate TCM with Western biomedicine? Are we in danger of taking the ‘traditional’ out of TCM?*”

1.5.5 Can functional genomic techniques overcome basic problems in *in vitro* research on phytocomplexes, and how?

Most members agree on the help that can come from functional genomics in *in vitro* research on phytocomplexes, both for the powerful tools used and for the more global vision of the biological system, but also for the help in experimental reproducibility: M1 “*could help a lot in the control of reproducibility of the systems, among to shed light into mechanisms involved in TCM in vitro effects*”; M5 “*A combination of omics and conventional methodologies should find its use in controlling the quality of research materials, suggesting both toxic and therapeutic effects*”; M4 “*All omics and conventional methodologies could help for to study both toxic and therapeutic profile of TCM materials*”; M2 “*help in addition with more conventional cell and molecular biological techniques to get a clearer picture*”; M9 “*the answer must be ‘yes’.....in silico ‘chemogenomics’ is, I think, equally valid*”; M7 “*functional genomic techniques have the advantage that with them the researchers get access to the “full-scale picture” of all changes that are happening after CHM treatment..... Based on the outcome of the functional genomic techniques the researchers could focus stronger their efforts in investigation of selected signaling pathways that are affected from the investigated CHMs*”; M8 “*A more global vision of the biological systems in their complexity and interconnections can help..... The use of a functional genomics approach, which allows to look at many different molecular targets within the same experimental complex, needs to be driven towards a view of the whole and not the simple sum of many different molecular variations.One important issue which cannot be addressed by the use of functional genomics is linked to the variability of the phytocomplex itself, leading to poor standardization, even though, thanks to DNA fingerprinting techniques and metabolomic characterization of plant material is now possible to obtain reference standards. This can certainly help, but still does not address the issue of reducing sample variability*”.

One member stressed that functional genomics does not solve possible misinterpretations coming from in vitro results with phytocomplexes: M10 “*It helps but the results may be misleading if it is not known how the phytocomplex is absorbed and metabolized etc in vivo*”

Unlike the others one member expressed a negative judgement on the issue: M3 “*No. I do not think so*”.

1.5.6 To what extent are functional genomic techniques used among researchers in the CHM field?

For this particular issue a PubMed search performed on March 30th 2011 (using the Boolean search construct: (“functional genomics*” or metabolomic* or metabonomic* or proteomic* or genomic* or “systems biology”) and (herbal or plant) gave a total of 13476 papers. When the term (Chinese or TCM) was added, the number reduced to 695, and adding (*in vitro*) resulted in just 26 papers. This clearly shows that there is still only very modest use of these techniques in the field, with no evidence of increasing usage or of initiatives to drive increased usage. The fact that there are so few *in vitro* papers is probably linked to the fact that it is not necessary to use an *in vitro* model to apply functional genomics analysis, which in fact can be performed more easily and usefully using human samples. This might in the future give a contribution to the next evolution in *in vitro* research.

In line with the results from the PubMed search most members do not think that the use of functional genomics is widespread at all among researchers in the CHM field: M1 “there are not much papers showing genomic analysis in TCM...”; M2 “*Currently the use is, as far as I can judge this, limited to a few groups*”; M5 “*As far as I know, not many. For example, to learn how many papers have studied CHM using “omics” and microarray technology, we searched Pubmed using the following searching strategies and only fewer than 166 papers were found (as of 25th March 2011): (gene array [mesh] OR genomics [mesh] OR DNA microarray [mesh] OR Oligonucleotide Array Sequence Analysis [mesh] OR metabolomics [mesh] OR proteomics [mesh] AND (Drugs, Chinese Herbal [mesh] OR Medicine, Chinese Traditional [mesh] OR Medicine, Oriental Traditional [mesh])*”; M8 “*despite their potential, their use is very limited*”; M9 “*Not a lot at present*”; M10 “*I suppose not much used*”.

Also, some of the members express mixed feelings: M3 “*I am not convinced that it will add considerable robust knowledge*”; M4 “*The application functional genomic techniques in CHM is an area of big promise research*”; M7 “*The application functional genomic techniques in CHM is still an area of research, which however is of big promise*”

1.5.7 What are the main obstacles for the use of a functional genomics approach by researchers in the CHM field?

Several members have stressed the limited expertise of researchers working in the field of CHM: M5 “*the poor knowledge about the affordability, promises and pitfalls of using these technologies*”; M7 “*...it needs a strong expertise...*”; M8 “*lack of expertise in functional genomics by many herbal in vitro researchers*”; M10 “*low expertise and knowledge in general*”. As a consequence many indicate that interdisciplinary collaboration among scientists from different fields, provides an answer to this problem: M1 “*most of the TCM scientist could find experts or platforms to be help with the genomic performance, even*

companies can do it"; M2 "collaborations with bio-informatics needed"; M7 "To successfully integrate the complexity of CHM with functional genomics approaches which are also very complex and often yield results that are difficult to interpret, it needs a strong expertise and joint work of scientists from many different fields of research: experts in TCM, phytochemists, experts in molecular biology/pharmacology/medicine, and often even informatics (since the evaluation and interpretation of the huge amount of data generated with the functional genomics approaches often is not very straight-forward)". The second major problem seems to be poor funding and technology availability: M4 "Probably funding"; M5 "Funding is the key issue and accessibility to core omics facilities and related bioinformatics services"; M8 "difficulties in finding equipment available for in vitro research – in general, public funding is rather limited in western countries for traditional medicine studies..."; M10 "Probably funding". Another problem is the complexity of experimental data and their interpretation: M2 "Data evaluation and interpretation → the production of really conclusive meaningful results"; M3 "the high extent of complexity which ask for "priorisation"; M5 "the huge amount of information these technologies could generate also switch researchers off". Two members also mention the problems with the experimental data which might be "patchy": M9 "for in silico chemogenomics. Here the main obstacles are patchy data (herbal compounds, biological activities, relevant PDB entries etc.)", or too variable: M1 "quality control should be assured"

1.5.8 How can *in-silico* tools be best applied to CHM research?

Unlike with the other questions, only 7 out of ten members gave answers to those on *in silico* tools. This by itself suggests the distance for many from the field of *in silico* and bio-informatics, which plays a pivotal role today, especially for interpreting experimental data from functional genomics. Indeed 5 members stress such roles: M1 "...useful for interpretation of omics results"; M2 "...mandatory use of bio-informatics in data evaluation/interpretation"; M7 "For evaluation and interpretation of the results obtained from functional genomics approaches"; M8 "in silico tools and more in general bioinformatics/computer biology are just essential for functional genomics application, both for multiple data analysis and systems modelling. Without their analytical power would be impossible to approach such complex experimental tools"; M9 "...even in the event that many targets of many compounds can be predicted with reasonable accuracy, there remains the question of how best to use that data. This requires input from bioinformatics (gene/disease relationships), and also TCM. Bridging the gulf between medical paradigms is something to which data mining is well suited".

The "classical" uses of *in silico* tools for modelling, for directing towards targets and for screening are also pointed out: M1 "they can be used as predictive tools for pointing out or directing molecular targets or mechanisms involved in TCM actions. They might narrow the amount of targets to be checked"; M2 "in silico tools such as pharmacophore modeling and in



in silico screening may be used to get first ideas about biological targets...”; M5 “in silico tools could be useful in finding patterns, pathways, effector/mediator clusters and help forge new hypothesis and guide future research”; M6 “in silico tools are very useful in the first steps of pharmacological research. Computational approaches allow us to evaluate a lot of molecules on several targets with a minimal economic effort”.

1.5.9 Are in silico approaches readily available and what would be needed to potentiate their application in TCM research?

In silico tools are considered rather available: M1 “Today, In silico approaches are mostly available for most of the scientist”; M5 “in silico approach is been used all the time in such an Information Technology era. For example, analysis of conventional and “omics” data are routinely carried out using computerised tools worldwide”; M7 “In silico approaches and tools that facilitate and complement molecular biology studies are very dynamic research area that quickly develops” . Some of the available tools though are not of good quality, so attention should be paid when using them: M5 “There are also many databases with specialised analysing powers, which are developed in the past years, but they are usually poorly maintained, updated and their accessibility is generally poor..... Sustainable support from governments, charities and the industrial community is needed for establishing authoritative and well-maintained systems; reliable multiple language translation is another on our wish list; open access or easy access will also make a great difference”; . Most answers stress the need for expertise and/or interdisciplinary collaborations to ensure use of in silico approaches: M1 “....good use and successful results from their use required a strong knowledge of them. To get results from in silico approaches, the scientific question to be solved should be accurately formulated to avoid getting tons of non-usable or non-interpretable information”; M2 “strong collaborations are needed”; M5 “there is much duplication in efforts, re-spinning the wheels and wasting limited resources”; M6 “The use/implementation of in silico approaches require specific expertises in computer science and computational chemistry, since the risk of producing biased results is not null”; M7 “Direct interaction with experts from that area is still is the best option to maximise the value of the obtained in silico predictions”; M8 “what is really needed is a better crosstalk between the in vitro and the in silico experts as well as an improved academic curriculum for in vitro scientists in order for them to better understand the language and the potentialities of computer science for their research”.

The answer from Member 9 can efficiently summarize everyday problems with the use of *in silico* tools, and is presented in its entirety:

M9: Most of the tools that have been developed so far either revolve around data (generally fairly simple inventories of relevant compounds) and docking (eg. TarFisDock and



iScreening). iScreening will apparently dock 20,000 compounds from CHM against any PDB receptor which the user uploads, and send you a list of the best 200 compounds.

The main difficulties are that many of the databases are difficult to use or access. The efficacy of the current docking tools is also questionable (in terms of speed, reliability etc.). Though they are, for the most part, easy to operate, they are not suitable for those with no background in virtual screening, and the results are intended for individual use (though there is probably no reason why they can't be collected by the provider in the form of a database). However, as yet, here is no centralized repository which combines information both on compounds and targets, traditional uses and so on, which can be easily accessed by users from a wide variety of backgrounds. So far, it is therefore the case that in silico approaches are at a fairly low level of organization/sophistication. Raw data and docking results for a limited number of compounds and/or targets comprise the first stage. There are further layers of analysis that need to be undertaken before a more complete picture begins to emerge. As mentioned above, these include bioinformatics and TCM.

Many of the appropriate tools are now in place, though refinements to most of them will continue to be made. That may not be as important as the application of different methods of virtual screening to compound databases (e.g. ligand-based, structure-based pharmacophores and docking), and the use of results based on consensus (which should become progressively easier).



ANNEX 1

Questionnaires returned from each member.

Member N.1

QUESTIONNAIRE ON THE USE OF FUNCTIONAL GENOMIC TECHNIQUES AND IN SILICO TOOLS FOR IN VITRO CHM RESEARCH

Name of WP4 member/s:

M Laura García Bermejo
.....

Other non member participants (optional):
.....
.

QUESTION 1:

- **What should be a correct approach to investigate molecular mechanism of action\molecular targets in CHM research?**

ANSWER 1:

In my opinion, it should be a mix of Molecular Biology Techniques. Before to consider the technique, on important question to address is the reproducibility of the studied systems, in terms of two important aspects: the use of a herbal mix, which components could vary from one to other preparations and the quality of each component of the mix in terms of contaminants or proportions of different compounds. As much as we controlled the herbal/single compounds to use, as better reproducibility of the results we will get. Synergism or antagonism between herbal compounds should be taken into account. Gen expression analysis are appropriated (gene signature) but higher levels of gene expression control such as miRNAs should not be forgetting.



Posttranslational protein modification analysis could be also pertinent. Finally, functional studies *in vivo* and *in vitro* modulating the molecular targets identified should be performed as a final proof of correct target identification..

QUESTION 2:

- **Can a Systems Biology approach overcome some of the problems hampering a full scale introduction of CHM *in vitro* research in the scientific mainstream?**

ANSWER 2:

In my opinion, apart from the fact that molecular techniques could reveal TCM molecular mechanisms of action, system biology approaches could be a powerful tool for herbal mixture quality control and standardization of TCM treatments, that, in my personal opinion is one of the main walls to be knocked down for TCM research

QUESTION 3:

- **In TCM *in vitro* research rank the following technique in terms of usefulness (explain the choice of the top ranking techniques)**
- Proteomics
- Metabolomics
- Genomics
- Other –omic techniques
- *In silico* models

ANSWER 3:

Depends what you want to study.

For quality control and setting up of robust *in vitro* and *in vivo* models of



study, genomics (very useful for *in vitro* studies), proteomics and metabolomics (necessary for *in vivo* models)

For further analyses of molecular mechanisms of action, we should also applied *in silico* models and other omic and non-omic techniques. 1.

QUESTION 4:

- **What are the main bottlenecks in this field?**

ANSWER 4:

The quality control of the herbs/mixtures/single compounds we will applied in experimental models

QUESTION 5:

- **Can functional genomic techniques overcome basic problems in *in vitro* research on phytocomplexes, and how?**

ANSWER 5:

As I mentioned before, these techniques could help a lot in the control of reproducibility of the systems, among to shed light into mechanisms involved in TCM *in vitro* effects.

QUESTION 6:

- **To what extent are functional genomic techniques used among researchers in the CHM field?**

ANSWER 6:

Up to my knowledge, they are not much papers showing genomic analysis in TCM. It seems that more articles are published applying functional genomics studies *in vitro* than *in vivo*. In any case, not many of them are reliable since quality control of the herbs and reproducibility of the systems used for performing studies are guaranteed.



QUESTION 7:

- **What are the main obstacles for the use of a functional genomics approach by researchers in the CHM field?**

ANSWER 7:

Technically, I do not see big problem, since most of the TCM scientist could find experts or platforms to be help with the genomic performance, even companies can do it. But, once again, quality control should be assured.

QUESTION 8:

- **How can *in silico* tools be best applied to CHM research?**

ANSWER 8:

In my opinion, they can be used as predictive tools for pointing out or directing molecular targets or mechanisms involved in TCM actions. They might narrow the amount of targets to be checked. Additionally, they are useful for interpretation of omics results.

QUESTION 9:

- **Are *in silico* approaches readily available and what would be needed to potentiate their application in TCM research?**

ANSWER 9:

Today, *in silico* approaches are mostly available for most of the scientist, although good use and successful results from their use required a strong knowledge of them. On the other hand, to get results from *in silico* approaches, the scientifically question to be solved should be accurately formulated to avoid getting tons of non-usable or non-interpretable information. Finally, *in silico* results must be confirmed by other molecular and cellular approaches.



Member N.2



QUESTIONNAIRE ON THE USE OF FUNCTIONAL GENOMIC TECHNIQUES AND IN SILICO TOOLS FOR IN VITRO CHM RESEARCH

Name of WP4 member/s:

...Verena Dirsch.....

Other non member participants (optional):

.....
.

QUESTION 1:

- **What should be a correct approach to investigate molecular mechanism of action\molecular targets in CHM research?**

ANSWER 1:

I think it is not possible to define the exact molecular mechanism or to identify the ultimate target(s) of compound mixtures. At best we can describe multiple effects that are mediated by this mixture (which is indeed sometimes "sold" as molecular mechanism).

QUESTION 2:

- **Can a Systems Biology approach overcome some of the problems hampering a full scale introduction of CHM in vitro research in the scientific mainstream?**

ANSWER 2:



Once a considerable amount of really meaningful data are published the acceptance in the scientific community will probably rise. Though I would not expect to become CHM scientific mainstream.

QUESTION 3:

- **In TCM in vitro research rank the following technique in terms of usefulness? (explain the choice of the top ranking techniques)**
- Proteomics
- Metabolomics
- Genomics
- Other –omic techniques
- *In silico* models

ANSWER 3: from Genomics to Proteomics it is getting more complex. Thus, the difficulties of an overall meaningful interpretation will probably rise from Genomics to Proteomics. On the other hand, these techniques should be even combined to get a meaningful picture of CHM effects. Without professional bio-informatics the outcome would be most likely limited.

Metabolomics/metabolic profiling (of plant constituents) in combination with functional assays may help in the field of quality control.

In silico tools can be extremely useful to find a direction where to go, but cannot be used a stand-alone technique.

QUESTION 4:

- **What are the main bottlenecks in this field?**

ANSWER 4:



Data evaluation and interpretation → the production of really conclusive meaningful results; collaborations with bio-informatics needed

QUESTION 5:

- **Can functional genomic techniques overcome basic problems in *in vitro* research on phytocomplexes, and how?**

ANSWER 5:

At least they may complement our tools and help in addition with more conventional cell and molecular biological techniques to get a clearer picture.

QUESTION 6:

- **To what extent are functional genomic techniques used among researchers in the CHM field?**

ANSWER 6:

Currently the use is, as far as I can judge this, limited to a few groups.

QUESTION 7:

- **What are the main obstacles for the use of a functional genomics approach by researchers in the CHM field?**

ANSWER 7:

Data evaluation and interpretation → the production of really conclusive meaningful results; collaborations with bio-informatics needed

QUESTION 8:

- **How can *in silico* tools be best applied to CHM research?**

ANSWER 8:

Next to the mandatory use of bio-informatics in data evaluation/interpretation, *in silico* tools such as pharmacophore modeling



and *in silico* screening may be used to get first ideas about biological targets (If there is any knowledge about the chemical structure of single compounds available)

QUESTION 9:

- **Are *in silico* approaches readily available and what would be needed to potentiate their application in TCM research?**

ANSWER 9:

Here strong collaborations are needed. And collaborations are only readily available when the scientific field attracts expert in this field.



Member N.3

QUESTIONNAIRE ON THE USE OF FUNCTIONAL GENOMIC TECHNIQUES
AND IN SILICO TOOLS FOR IN VITRO CHM RESEARCH

Name of WP4 member/s:

Vollmar Angelika
.....

Other non member participants (optional):
.....
.

QUESTION 1:

- **What should be a correct approach to investigate molecular mechanism of action\molecular targets in CHM research?**

ANSWER 1:

A combination of classical
biochemical signaling work and combination with “state of the art” target
fishing procedure, live cell imaging, and –omics technology

QUESTION 2:

- **Can a Systems Biology approach overcome some of the problems hampering a full scale introduction of CHM in vitro research in the**



scientific mainstream?

Yes of course, however the bioinformatic analysis is of major importance

ANSWER 2:

QUESTION 3:

- **In TCM *in vitro* research rank the following technique in terms of usefulness? (explain the choice of the top ranking techniques)**
- Proteomics 1
- Metabolomics 2
- Genomics 4
- Other –omic techniques 5
- *In silico* models 3

ANSWER 3:

QUESTION 4:

- **What are the main bottlenecks in this field?**

ANSWER 4:

Complexity and its meaningful interpretation

QUESTION 5:

- **Can functional genomic techniques overcome basic problems in *in vitro* research on phytocomplexes, and how?**

ANSWER 5:



No. I do not think so



QUESTION 6:

- **To what extent are functional genomic techniques used among researchers in the CHM field?**

ANSWER 6:

To a high extent, however I am not convinced that it will add considerable Robust knowledge

QUESTION 7:

- **What are the main obstacles for the use of a functional genomics approach by researchers in the CHM field?**

ANSWER 7:

As I said the high extent of complexity which ask for “priorisation”

QUESTION 8:

- **How can *in-silico* tools be best applied to CHM research?**

ANSWER 8:

Sorry I am not an expert in this filed

QUESTION 9:

- **Are *in silico* approaches readily available and what would be needed to potentiate their application in TCM research?**

ANSWER 9:

I apologize again – for not having a competent answer



Member N.4

QUESTIONNAIRE ON THE USE OF FUNCTIONAL GENOMIC TECHNIQUES AND IN SILICO TOOLS FOR IN VITRO CHM RESEARCH

Name of WP4 member/s:

Maria Carrara

Other non member participants (optional):

.....
.

QUESTION 1:

- **What should be a correct approach to investigate molecular mechanism of action\molecular targets in CHM research?**

ANSWER 1: A combination of conventional molecular biology and other advanced technique

QUESTION 2:

- **Can a Systems Biology approach overcome some of the problems hampering a full scale introduction of CHM in vitro research in the scientific mainstream?**

ANSWER 2: May be, but more evidence is needed



QUESTION 3:

- **In TCM *in vitro* research rank the following technique in terms of usefulness? (explain the choice of the top ranking techniques)**
- Proteomics
- Metabolomics
- Genomics
- Other –omic techniques
- *In silico* models

ANSWER 3: It depends on what you want to demonstrated. If the transcriptional regulation is the main hypothesis, genomics have been shown sensitive and specific; in the case you would study biological processes involving proteins , proteomic should be used; if you would study an effect on metabolism, metabolomics is the first choice

QUESTION 4:

- **What are the main bottlenecks in this field?**

ANSWER 4: The variability of the starting material

QUESTION 5:

- **Can functional genomic techniques overcome basic problems in *in vitro* research on phytocomplexes, and how?**

ANSWER 5: All -omics and conventional methodologies could help for to



study both toxic and therapeutic profile of TCM materials.

QUESTION 6:

- **To what extent are functional genomic techniques used among researchers in the CHM field?**

ANSWER 6: The application functional genomic techniques in CHM is an area of big promise research.

QUESTION 7:

- **What are the main obstacles for the use of a functional genomics approach by researchers in the CHM field?**

ANSWER 7: Probably funding

QUESTION 8:

- **How can *in silico* tools be best applied to CHM research?**

ANSWER 8: No experience in the field

QUESTION 9:

- **Are *in silico* approaches readily available and what would be needed to potentiate their application in TCM research?**

ANSWER 9: No experience in the field



Member N.5

QUESTIONNAIRE ON THE USE OF FUNCTIONAL GENOMIC TECHNIQUES
AND IN SILICO TOOLS FOR IN VITRO CHM RESEARCH

Name of WP4 member/s:

Qihe Xu.....

Other non member participants (optional):

.....
.

QUESTION 1:

- **What should be a correct approach to investigate molecular mechanism of action\molecular targets in CHM research?**

ANSWER 1: Ideally, a combination of conventional molecular biology methods, “omics” technology, bioinformatics, systems biology and other emerging technologies.

QUESTION 2:

- **Can a Systems Biology approach overcome some of the problems hampering a full scale introduction of CHM in vitro research in the scientific mainstream?**



ANSWER 2: Possible, but more evidence is clearly needed.

QUESTION 3:

- **In TCM in vitro research rank the following technique in terms of usefulness? (explain the choice of the top ranking techniques)**
- Proteomics
- Metabolomics
- Genomics
- Other –omic techniques
- *In silico* models

ANSWER 3:

It depends on your hypothesis and the individual applications.

For a hypothesis focusing on transcriptional regulation, genomic/transcriptomic tools have been shown to be both sensitive, specific and affordable; for biological processes in which proteins and peptides are of the main concern, proteomics should be used, although it might not be as sensitive and specific as microarray yet; finally, if the metabolic profile is the main concern, metabolomics is the first choice. Ideally, a combination of multiple technologies including omics, conventional molecular biology technology, activity and/or functional assays and imaging tools could be most powerful.



QUESTION 4:

- **What are the main bottlenecks in this field?**

ANSWER 4: Although all “omics” technologies have been more sensitive, specific, powerful and affordable than ever before, the current funding to TCM and other herbal medicine research in Europe is lacking, making it the top bottleneck to bring these powerful technologies in research of complex preparations such as Chinese herbal medicines.

QUESTION 5:

- **Can functional genomic techniques overcome basic problems in *in vitro* research on phytocomplexes, and how?**

ANSWER 5: A combination of omics and conventional methodologies should find its use in controlling the quality of research materials, suggesting both toxic and therapeutic effects, as well as defining mechanisms of actions and signaling pathways, etc.

QUESTION 6:

- **To what extent are functional genomic techniques used among researchers in the CHM field?**

ANSWER 6:

As far as I know, not many. For example, to learn how many papers have studied CHM using “omics” and microarray technology, we searched Pubmed using the following searching strategies and only fewer than 166 papers were found (as of 25th March 2011): (gene array [mesh] OR genomics [mesh] OR DNA microarray [mesh] OR Oligonucleotide Array Sequence Analysis [mesh]



OR metabolomics [mesh] OR proteomics [mesh] AND (Drugs, Chinese Herbal [mesh] OR Medicine, Chinese Traditional [mesh] OR Medicine, Oriental Traditional [mesh])

QUESTION 7:

- **What are the main obstacles for the use of a functional genomics approach by researchers in the CHM field?**

ANSWER 7:

Funding is the key issue. In addition, accessibility to core omics facilities and related bioinformatics services, the poor knowledge about the affordability, promises and pitfalls of using these technologies and the fears of the huge amount of information these technologies could generate also switch researchers off.

QUESTION 8:

- **How can *in silico* tools be best applied to CHM research?**

ANSWER 8:

Built on ever –strengthening bioinformatics technologies, *in silico* tools could be useful in finding patterns, pathways, effector/mediator clusters and help forge new hypothesis and guide future research.

QUESTION 9:

- **Are *in silico* approaches readily available and what would be needed to potentiate their application in TCM research?**

ANSWER 9:



I think *in silico* approach is being used all the time in such an Information Technology era. For example, analysis of conventional and “omics” data are routinely carried out using computerised tools worldwide. There are also many databases with specialised analysing powers, which are developed in the past years, but they are usually poorly maintained, updated and their accessibility is generally poor. Meanwhile there is much duplication in efforts, re-spinning the wheels and wasting limited resources.

Sustainable support from governments, charities and the industrial community is needed for establishing authoritative and well-maintained systems; reliable multiple language translation is another on our wish list; open access or easy access will also make a great difference.



Member N.6

QUESTIONNAIRE ON THE USE OF FUNCTIONAL GENOMIC TECHNIQUES AND IN SILICO TOOLS FOR IN VITRO CHM RESEARCH

Name of WP4 member/s:

.....

Other non member participants (optional):

Ivano Eberini

QUESTION 1:

- **What should be a correct approach to investigate molecular mechanism of action\molecular targets in CHM research?**

ANSWER 1:

A correct procedure should consist in a well-consolidated pipeline composed of different approaches:

- *In silico* evaluation of putative ligands on different targets
- *In vitro* validation of the positive hits through cellular molecular pharmacology assays
- *In vivo* validation of wet results on animal models for the investigated pathologies



QUESTION 2:

- **Can a Systems Biology approach overcome some of the problems hampering a full scale introduction of CHM in vitro research in the scientific mainstream?**

ANSWER 2:

In my opinion, systems biology is not useful for overcoming specific problems. It should be implemented in the final stages of the research for networking all the data collected during the previous steps.

QUESTION 3:

- **In TCM in vitro research rank the following technique in terms of usefulness? (explain the choice of the top ranking techniques)**
- Proteomics
- Metabolomics
- Genomics
- Other –omic techniques
- *In silico* models

ANSWER 3:

No usefulness ranking should be done. All these techniques should be implemented according to a specific time of the research:

1. *In silico* models
2. Proteomics
3. Metabolomics
4. Genomics



QUESTION 4:

- **What are the main bottlenecks in this field?**

ANSWER 4:

Several of the targets of drugs/active molecules are proteins. The identification of potential targets involved in specific pathologies, and the knowledge of their structures are difficult to obtain but necessary for all the subsequent steps.

QUESTION 5:

- **Can functional genomic techniques overcome basic problems in *in vitro* research on phytocomplexes, and how?**

ANSWER 5:

I don't know.

QUESTION 6:

- **To what extent are functional genomic techniques used among researchers in the CHM field?**

ANSWER 6:

I don't know.

QUESTION 7:

- **What are the main obstacles for the use of a functional genomics approach by researchers in the CHM field?**

ANSWER 7:

I don't know.

QUESTION 8:

- **How can *in silico* tools be best applied to CHM research?**



ANSWER 8:

The *in silico* tools are very useful in the first steps of pharmacological research. Computational approaches allow us to evaluate a lot of molecules on several targets with a minimal economic effort. The positive hits can subsequently be investigated through *in vitro* and/or *in vivo* approaches, which are more expensive and associated to a lower throughput power.

QUESTION 9:

- **Are *in silico* approaches readily available and what would be needed to potentiate their application in TCM research?**

ANSWER 9:

The use/implementation of *in silico* approaches require specific expertises in computer science and computational chemistry, since the risk of producing biased results is not null. An efficient application of *in silico* approaches requires the chemical characterization of putative active compounds and their organization in chemical/structural databases.



Member N.7

QUESTIONNAIRE ON THE USE OF FUNCTIONAL GENOMIC TECHNIQUES AND IN SILICO TOOLS FOR IN VITRO CHM RESEARCH

Name of WP4 member/s:

Atanas Atanasov

.....

Other non member participants (optional):

.....

.

QUESTION 1:

- **What should be a correct approach to investigate molecular mechanism of action\molecular targets in CHM research?**

ANSWER 1:

To achieve reliable identification of the molecular targets and the active compounds/mixtures of compounds that mediate the CHM action, a very important and often underestimated factor is thorough quality control of the starting material. Furthermore, it seems reasonable to well keep in mind the possibility that the final outcome could be due to a synergistic or additive action of different chemical constituents present in the crude herbal drug. Before trying to identify the molecular targets mediating the action of the CHM, the most straight-forward approach would be first to identify single compounds, or mixtures of well defined single compounds that would have similar effectiveness as the complex crude drug in respect to a reliable functional readout. Having single compounds or well defined mixtures with known composition would assure reliability and reproducibility of the findings, and next step than would be to attempt to identify the single molecular target(s) that account for the observed functional effect.



QUESTION 2:

- **Can a Systems Biology approach overcome some of the problems hampering a full scale introduction of CHM in vitro research in the scientific mainstream?**

ANSWER 2:

The complexity of CHM is a very big challenge, which makes it very difficult to track the main molecular targets and mechanisms mediating CHM action. Systems Biology approaches have the advantage that with them the researchers get access to the “full-scale picture” of all changes that are happening after CHM treatment, which might give valuable clues about the main affected signaling events and pathways. Knowledge for the main affected signaling pathways would then greatly facilitate a more focused research effort aiming to reveal from one side the single active compounds of the CHM, and from other side the single affected molecular targets within the affected pathway.

QUESTION 3:

- **In TCM in vitro research rank the following technique in terms of usefulness? (explain the choice of the top ranking techniques)**
- Proteomics
- Metabolomics
- Genomics
- Other –omic techniques
- *In silico* models

ANSWER 3:



1. Genomics (most useful);
2. Proteomics;
3. Metabolomics;
4. *In silico* models;
5. Other –omic techniques.

The readout obtained with Genomics is less complicated and therefore might represent a better alternative than Proteomics and Metabolomics. Genomics is just taking account for differences in gene expression, whereas Proteomics readout is influenced on further events such as alternative splicing, rate of translation, posttranslational modifications etc. which makes it more complicated. Metabolomics has also a further degree of complexity, because it is not influenced just from gene expression and from protein expression, but also from external influences like for example the dietary intake of the respective metabolite(s). To investigate such a complex drugs such as CHM, it might represent a good choice to start with System biology approach that is possibly simpler and more upstream (in that case Genomics). *In silico* models are rated lower than the three –omics approaches, since most often nowadays the *in silico* approaches are still having too low predictive power, and the value of the real experimental data is still much bigger.

QUESTION 4:

- **What are the main bottlenecks in this field?**

ANSWER 4:

The variability of the quality of the starting material, which impairs the reproducibility of the obtained scientific results.



QUESTION 5:

- **Can functional genomic techniques overcome basic problems in *in vitro* research on phytocomplexes, and how?**

ANSWER 5:

As already outlined in Answer 2, functional genomic techniques have the advantage that with them the researchers get access to the “full-scale picture” of all changes that are happening after CHM treatment, which might give valuable clues about the main affected signaling events and pathways. Based on the outcome of the functional genomic techniques the researchers could focus stronger their efforts in investigation of selected signaling pathways that are affected from the investigated CHMs.

QUESTION 6:

- **To what extent are functional genomic techniques used among researchers in the CHM field?**

ANSWER 6:

The application functional genomic techniques in CHM is still an area of research, which however is of big promise.

QUESTION 7:

- **What are the main obstacles for the use of a functional genomics approach by researchers in the CHM field?**

ANSWER 7:

To successfully integrate the complexity of CHM with functional genomics approaches which are also very complex and often yield results that are difficult to interpret, it needs a strong expertise and joint work of scientists from many different fields of research: experts in TCM, phytochemists, experts in molecular biology/pharmacology/medicine, and often even informatics (since the evaluation and interpretation of the huge amount of data generated with the functional genomics approaches often is not very



straight-forward).



QUESTION 8:

- **How can *in silico* tools be best applied to CHM research?**

ANSWER 8:

For evaluation and interpretation of the results obtained from functional genomics approaches.

QUESTION 9:

- **Are *in silico* approaches readily available and what would be needed to potentiate their application in TCM research?**

ANSWER 9:

In silico approaches and tools that facilitate and complement molecular biology studies are very dynamic research area that quickly develops. Direct interaction with experts from that area is still is the best option to maximise the value of the obtained *in silico* predictions.



Member N.8

QUESTIONNAIRE ON THE USE OF FUNCTIONAL GENOMIC TECHNIQUES AND IN SILICO TOOLS FOR IN VITRO CHM RESEARCH

Name of WP4 member/s:

Alessandro Buriani.....

Other non member participants (optional):

.....

QUESTION 1:

- **What should be a correct approach to investigate molecular mechanism of action\molecular targets in CHM research?**

ANSWER 1: A deterministic approach to investigate molecular mechanism of action or molecular targets is nowadays showing its limits, and there is a growing consensus in the scientific community that focusing into single molecular contexts as if they were isolated entities has a very limited value for a correct approach to pharmaco-toxicological studies. This is particularly apparent when dealing with research on herbal drugs, where multiple active components are present. Today a Systems Biology approach allows us to get closer to a real view of the multiple and simultaneous effects exerted by phytocomplexes on biological systems. This is true also when using single active components, and in fact the complexity issue is still true when



considering single molecules, which directly or indirectly can affect multiple biological targets.

Using functional genomics techniques can allow to do initial screening directly on the molecular targets affected, for instance using DNA microarrays. Once identified, clusters of interrelated molecular targets can be investigated more in deep, using –omics techniques like proteomics or metabolomics. Basically, while previously the focus on the mechanism would be led by the observed biological effect, now, using high throughput analysis it is possible to reach directly that information in the very beginning of a research project.

QUESTION 2:

- **Can a Systems Biology approach overcome some of the problems hampering a full scale introduction of CHM in vitro research in the scientific mainstream?**

ANSWER 2:

Systems biology zooms out the focus from the detail to the system in its dynamic complexity. This approach allows an analysis of the mechanisms of action of active molecules on direct and indirect targets down to the changes of the biological homeostatic equilibrium. This is particularly important when testing phytocomplexes and especially formulations, like in CHM, given that each component can have multiple targets linked to the pharmacological and toxicological activity. A systems biology approach thus seems particularly fit for studying CHM, providing a strategy for collecting every detail of each system to merge them together in a complex or holistic



vision. Today with the same principle a novel medical approach is emerging, personalized medicine, where the subject is seen as a whole with all its molecular characteristics which distinguish it from the rest. This new vision, affecting both biology and medicine, is actually providing a special ground to overcome one of the main cultural problems for the diffusion of TCM in the western scientific cultural mainstream: a holistic vision versus a deterministic one. Indeed this contradiction has no reason to exist any longer in the era of Systems Biology, and this will give a fundamental contribution to the full scale introduction of CHM in the scientific mainstream.

QUESTION 3:

- **In TCM in vitro research rank the following technique in terms of usefulness? (explain the choice of the top ranking techniques)**
- Proteomics
- Metabolomics
- Genomics
- Other –omic techniques
- *In silico* models

ANSWER 3:

- 1 - Proteomics
- 1 – Metabolomics

Proteomics and metabolomics, allow a precise and complex observation of the downstream molecular effects, both direct and indirect, some of

which might be more relevant or evident on proteins and some others on metabolites, depending on the kind of global biological effect. So the two –omics can be considered equally relevant and might be applied more conveniently to different experimental contexts.

- 2 - *In silico* models. *In silico* models allow an efficient and rapid interpretation of experimental data to read their meaning in complex systems, which otherwise in many cases would not be possible. They can be essential to obtain a unique (holistic) vision of many different molecular effects. They can also be used for simulations which can speed up *in vitro* research.
- 3 – Genomics. DNA arrays can be used as a screening tool before proceeding towards techniques more focused on downstream effects. Another experimental use of genomics is herbal DNA fingerprinting which has a very high value in standardization of plant material.
- 4 - Other –omic techniques: though valuable, these techniques are still poorly used, so more work is needed to reach a more standard and shared view of these –omics in the scientific community

QUESTION 4:

- **What are the main bottlenecks in this field?**

ANSWER 4: though knowledge is fast growing in the field, its translation to student education programs is still not satisfactory. This education gap is rather tangible among research scientists and this slows down their approach to functional genomics application. Moreover, in the context of *in vitro* research, scientists have been using experimental models that do not allow functional genomics to express their full potential. An example is given by the use of single molecules or single cells, while functional genomics would allow us to look at entire biological systems. In CHM research where



in many cases it would be possible to use human samples like blood or urines, this is particular relevant, since it would permit it to be in close contact with the clinical use.

Many western scientists do not even get close to CHM research, also because is not officially used in many western countries and this gives it a low political priority at a single state level for financing purposes.

The other point which is also a direct consequence of the others is that few herbal research centers do actually have the possibility of conducting research using a functional genomics approach. The techniques require instruments and expertise which are hardly available for *in vitro* research.

QUESTION 5:

- **Can functional genomic techniques overcome basic problems in *in vitro* research on phytocomplexes, and how?**

ANSWER 5:

Some of the basic problems linked to the *in vitro* use of phytocomplexes are due to the limits of experimental models themselves, where only a biological effect or one of its molecular aspects are considered. A more global vision of the biological systems in their complexity and interconnections can help to overcome such experimental limit. The use of a functional genomics approach, which allows us to look at many different molecular targets within the same experimental complex, needs to be driven towards a view of the whole and not the simple sum of many different molecular variations. In this case using a phytocomplex might not be so different from using a single



molecule. One important issue which cannot be addressed by the use of functional genomics is linked to the variability of the phytochemical itself, leading to poor standardization of the experimental samples, even though, thanks to DNA fingerprinting techniques and metabolomic characterization of plant material is now possible to obtain reference standards. This can certainly help, but still does not address the issue of reducing sample variability.

QUESTION 6:

- **To what extent are functional genomic techniques used among researchers in the CHM field?**

ANSWER 6: despite their potential, their use is very limited.

QUESTION 7:

- **What are the main obstacles for the use of a functional genomics approach by researchers in the CHM field?**

ANSWER 7: lack of expertise in functional genomics by many herbal in vitro researchers – difficulties in finding equipment available for in vitro research –in general, public funding is rather limited in western countries for traditional medicine studies, projects using functional genomics instead require high costs especially in the start up phase, where new updated technology is needed.

QUESTION 8:

- **How can *in silico* tools be best applied to CHM research?**



ANSWER 8: *in silico* tools and more in general bioinformatics/computer biology are just essential for functional genomics application, both for multiple data analysis and systems modelling. Without their analytical power would be impossible to approach such complex experimental tools.

QUESTION 9:

- **Are *in silico* approaches readily available and what would be needed to potentiate their application in TCM research?**

ANSWER 9: what is really needed is a better crosstalk between the *in vitro* and the *in silico* experts as well as an improved academic curriculum for *in vitro* scientists in order for them to better understand the language and the potentialities of computer science for their research



Member N.9

QUESTIONNAIRE ON THE USE OF FUNCTIONAL GENOMIC TECHNIQUES
AND IN SILICO TOOLS FOR IN VITRO CHM RESEARCH

Name of WP4 member/s:

...Tom Ehrman.....

Other non member participants (optional):

.....
.

QUESTION 1:

- **What should be a correct approach to investigate molecular mechanism of action\molecular targets in CHM research?**

ANSWER 1: (a) *In silico* followed by *in vitro* approach. Computational models (docking, ligand-based, pharmacophores) first used to screen phytochemical libraries, followed by *in vitro* confirmation. (b) Molecular modeling used to investigate likely mode of action (e.g. docking) of *in vitro* results. For genomic-scale screening, approach (a) is the most feasible.

QUESTION 2:

- **Can a Systems Biology approach overcome some of the problems hampering a full scale introduction of CHM in vitro research in the**



scientific mainstream?

ANSWER 2: Yes. For example, in the case of signal transduction or metabolic pathways, network analysis may help to simplify the range of targets to be considered, in the first instance, by identifying critical components (eg. those showing high 'centrality').

On data sets containing large numbers of *in silico* predictions against a large battery of targets, then clustering of target fingerprints should also prove informative.

QUESTION 3:

- **In TCM *in vitro* research rank the following technique in terms of usefulness? (explain the choice of the top ranking techniques)**
- Proteomics
- Metabolomics
- Genomics
- Other –omic techniques
- *In silico* models

ANSWER 3: I would rank metabolomic and *in silico* approaches at the top. Metabolomics offers the most appealing approach both to identifying cellular responses to a particular CHM compound, and to understanding how that response is modified in the case of additional compounds (ie. one of the most revealing ways to study synergy). *In silico* approaches provide a 'theoretical' arm to the study of CHM, without which a unifying framework is that much more difficult.

QUESTION 4:

- **What are the main bottlenecks in this field?**

ANSWER 4: Many. (a) lack of information and appropriate databases (though much improved compared to several years ago); (b) lack of personnel with appropriate skills; (c) lack of funds (?). Another point – to what extent are efforts being made to integrate TCM with Western biomedicine? Are we in danger of taking the ‘traditional’ out of TCM?

QUESTION 5:

- **Can functional genomic techniques overcome basic problems in *in vitro* research on phytocomplexes, and how?**

ANSWER 5: I’m not an expert on many aspects of functional genomics, but I think the answer must be ‘yes’, as in what I said about metabolomics and herbal synergies (*in silico* ‘chemogenomics’ is, I think, equally valid in this respect, though approaching it from a different angle).

QUESTION 6:

- **To what extent are functional genomic techniques used among researchers in the CHM field?**

ANSWER 6: Not a lot at present as far as I’m aware.

QUESTION 7:

- **What are the main obstacles for the use of a functional genomics approach by researchers in the CHM field?**



ANSWER 7: I can only really answer for *in silico* chemogenomics. Here the main obstacles are patchy data (herbal compounds, biological activities (eg. for constructing or validating predictive models), relevant PDB entries etc.). There is also the question of appropriate software for high throughput screening, reliability of the approaches on which it is based, and the question of whether single models are really sufficient, in the case of *in silico* prediction, to justify basing *in vitro* studies around them.

QUESTION 8:

- **How can *in-silico* tools be best applied to CHM research?**

ANSWER 8: Chemogenomics may be the single most important objective. However, even in the event that many targets of many compounds can be predicted with reasonable accuracy, there remains the question of how best to use that data. This requires input from bioinformatics (gene/disease relationships), and also TCM. Bridging the gulf between medical paradigms is something to which data mining is well suited.

QUESTION 9:

- **Are *in silico* approaches readily available and what would be needed to potentiate their application in TCM research?**

ANSWER 9: Most of the tools that have been developed so far, either revolve around data (generally fairly simple inventories of relevant compounds) and docking (e.g. TarFisDock and iScreening). iScreening will apparently dock 20,000 compounds from CHM against any PDB receptor



which the user uploads, and send you a list of the best 200 compounds.

The main difficulties are that many of the databases are difficult to use or access. The efficacy of the current docking tools is also questionable (in terms of speed, reliability etc.). Though they are, for the most part, easy to operate, they are not suitable for those with no background in virtual screening, and the results are intended for individual use (though there is probably no reason why they can't be collected by the provider in the form of a database). However, as yet, there is no centralized repository which combines information both on compounds and targets, traditional uses and so on, which can be easily accessed by users from a wide variety of backgrounds.

So far, it is therefore the case that *in silico* approaches are at a fairly low level of organization/sophistication. Raw data and docking results for a limited number of compounds and/or targets comprise the first stage. There are further layers of analysis that need to be undertaken before a more complete picture begins to emerge. As mentioned above, these include bioinformatics and TCM.

Many of the appropriate tools are now in place, though refinements to most of them will continue to be made. That may not be as important as the application of *different* methods of virtual screening to compound databases (e.g. ligand-based, structure-based pharmacophores and docking), and the use of results based on consensus (which should become progressively easier).



Member N.10

QUESTIONNAIRE ON THE USE OF FUNCTIONAL GENOMIC TECHNIQUES AND IN SILICO TOOLS FOR IN VITRO CHM RESEARCH

Name of WP4 member/s:

Enrica Bosisio

Other non member participants (optional):

.....
.

QUESTION 1:

- **What should be a correct approach to investigate molecular mechanism of action\molecular targets in CHM research?**

ANSWER 1:

Any molecular biology method, conventional or advanced technique, it depends on the question you want to give an answer

QUESTION 2:

- **Can a Systems Biology approach overcome some of the problems hampering a full scale introduction of CHM in vitro research in the scientific mainstream?**

ANSWER 2:

May be, I would need more experience about this approach, to reply



QUESTION 3:

- **In TCM in vitro research rank the following technique in terms of usefulness (explain the choice of the top ranking techniques)**
- Proteomics
- Metabolomics
- Genomics
- Other –omic techniques
- *In silico* models

ANSWER 3:

It depends on what you want to demonstrate: if it is an effect on metabolism: metabolomics, an effect at gene level genomics etc. In case you would correlate gene transcription and protein levels, both transcriptomic and metabolomics.

QUESTION 4:

- **What are the main bottlenecks in this field?**

ANSWER 4:

Fund availability, and the transfer of in vitro results to in vivo situation: see my reply to question 5

QUESTION 5:

- **Can functional genomic techniques overcome basic problems in *in***



***vitro* research on phytocomplexes, and how?**

ANSWER 5:

It helps but the results may be misleading if it is not known how the phytocomplex is absorbed and metabolized etc in vivo. In other words: what you test in a well, is not what you find systemically.

QUESTION 6:

- **To what extent are functional genomic techniques used among researchers in the CHM field?**

ANSWER 6:

I would need to make a PubMed search, in order to reply. I suppose not much used

QUESTION 7:

- **What are the main obstacles for the use of a functional genomics approach by researchers in the CHM field?**

ANSWER 7:

Probably funding, but also low expertise and knowledge in general

QUESTION 8:

- **How can *in silico* tools be best applied to CHM research?**

ANSWER 8:

No reply: no experience in the field



QUESTION 9:

- **Are *in silico* approaches readily available and what would be needed to potentiate their application in TCM research?**

ANSWER 9:

No reply: no experience in the field