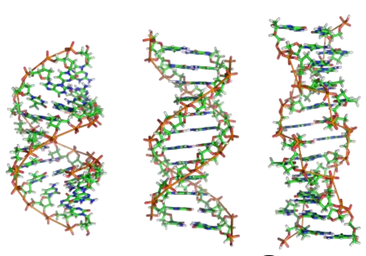
# Site-specific reaction of benzoquinone and plumbagin with the histone acetyltransferase CBP, CREB-binding protein identified by Quadrupole-Orbitrap LC-MS

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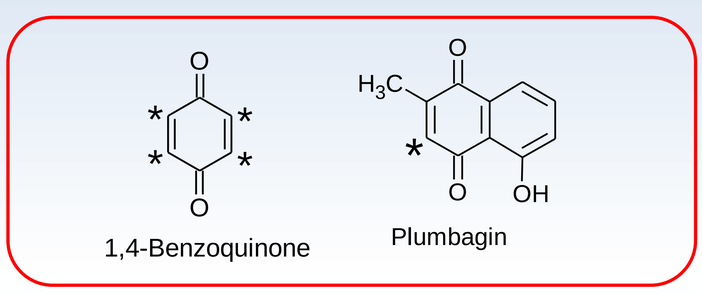
## Introduction

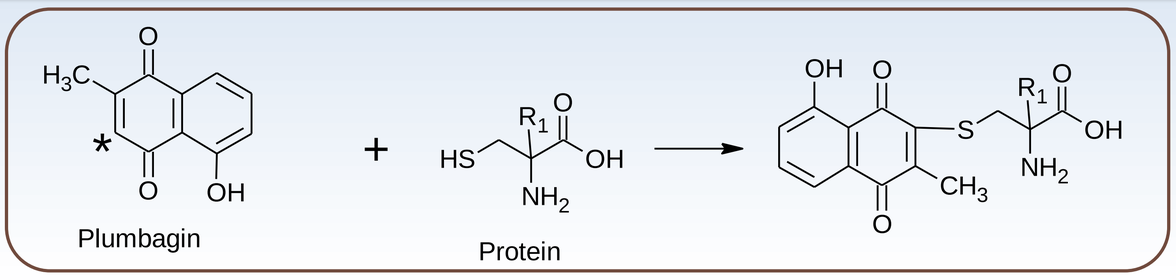
Quinones are molecules occurring in nature. These chemicals are found in most living organisms. In addtion, several quinones can also be of pharmacological and toxicological interest such as certain toxic quinones present in polluted air. Quinones have two main chemical properties: they can act as electrophiles and/or oxidants in oxido-reduction reactions.

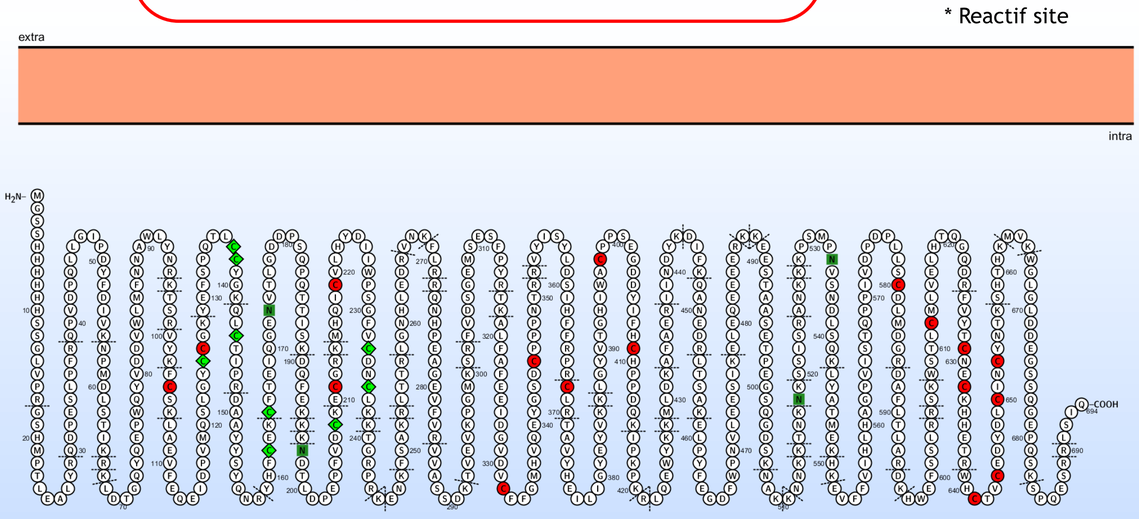
One of the main reaction of quinones is the alkylation of essential protein thiol or amine groups and/or their oxidation by reactive oxygen species generated through redox cycling of quinones. These two mechanisms are likely to explain the biological effects of certain quinones including benzoquinone and plumbagin.

In the present study, we identified cysteine residues in the CBP (CREB Binding Protein), a key lysine/histone acetyltransferase known to act as a transcriptional co-activator. We found that histone acetyltransferase is specifically modified by two well-known quinones (benzoquinone and plumbagin).

Interestingly, modifications of certain of these cysteines are common events in a number of pathologies and lead to the inactivation of the CBP acetyltransferase activity.




*Sequence of CBP, with the trypsin site, the cystein and identified cysteins (Protter - visualize proteoforms Omasits et al., Bioinformatics. 2013 Nov 21.)*

## Material and Methods

The redox state of cysteine residues of CBP exposed or not (CTL) to BQ/Plumbagin was determined by differential cysteine labelling and mass spectrometry analysis. The first alkylant is the N-Ethylmalmeide (NEM). The samples were then purified in polyacrylamide gel and a reduction with DTT is applied before the second alkylant iodoacetamide (CAM). The samples were digested by trypsin overnight at 37°C and were analysed by LC, directly coupled to QqOrbitrap mass spectrometer (Qexactive, Thermofisher Scientific)

## Database research, interpretation and quantification

The raw data were processed using Proteome Discover v.1.4 (ThermoFisher Scientific) with Mascot v.2.5 (Matrix Science) as search algorithm. Each sub-sample was first analyzed in triplicate. The database search was done on merged data using Mascot search engine on sequence of the recombinant protein. The following parameters were used: up to 2 missed cleavages; MS tolerance 10 ppm; MS/MS tolerance 20 ppm; carbamidomethylation (C), NEM(C), Plumbagin (+157, C) and oxidation (M) as partial modifications. The peptide modified by benzoquinone is not identified by mass spectrometry that is why we only have quantified the signal peptide between the sample treated and not treated.

## Results

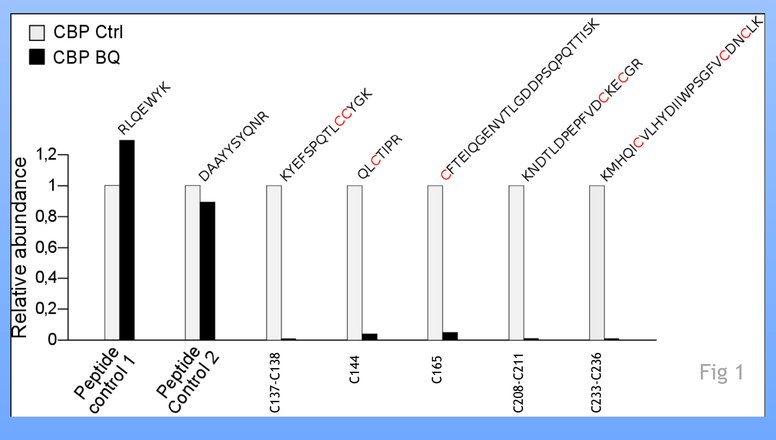
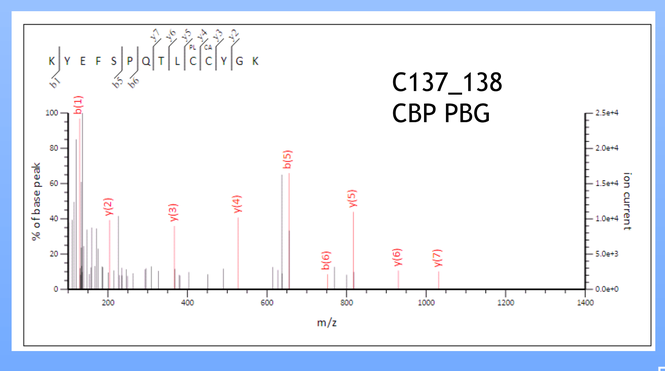



Fig 1



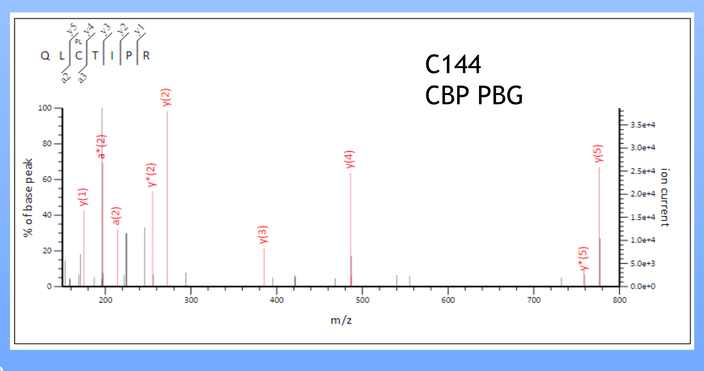




Fig 2

Fig 1 : Quantification of signal of peptides with a different cysteine residue between CTL and BQ
Fig 2: MS/MS spectra of the $MH^{3+}$ (m/z 637.61) PBG-alkylated peptide KYEFSPQTLCCYGK and MS/MS spectra of the $MH^{2+}$ (m/z 509.25) PBG-alkylated peptide QLCTIPR

## Conclusion

Mass spectrometry analysis indicates that certain quinones of toxicological and pharmacological interest react with specific cysteine residues of CBP which may impact the biological functions of this key histone acetyltransferase.