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Sometimes the most effective proteomics includes a lot of non-proteomics

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OUTLINE

I. Proteomics analysis of actions of a dietary supplement, grape seed extract (GSE), in mammalian brain;
II. FT-ICR MS mapping of HNE adduct sites on recombinant creatine kinase (CK-BB);
III. Enzyme assays: HNE stoichiometrically poisons CK-BB;
IV. Unexpected results from studies of CK-BB in AD brain

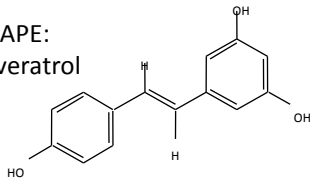
Our principal goal: to understand the molecular basis of human chronic conditions/diseases, to develop prevention or therapies.

Strategy: a ***proteomics*** approach

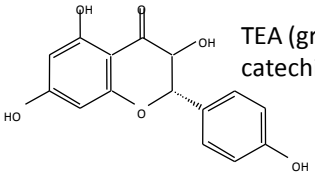
Hypothesis: Actions of "beneficial" agents such as dietary anti-oxidants in normal and disease tissue will reveal subproteomes of proteins "at risk" for disease-relevant changes.

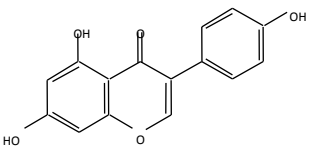
POLYPHENOLS: similar structures among themselves, and with 17β-estradiol

GRAPE: resveratrol

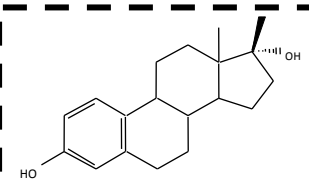


TEA (grapes): catechin





SOY: genistein



17β-estradiol

The experiment: give grapeseed powder in rat diet; examine brain proteomes

AIN-76A diet; normal adult rats

(n = 5)

AIN-76A + 5% GSE; normal adult rats
(n = 5)

whole brain homogenates, triplicate 2D gels

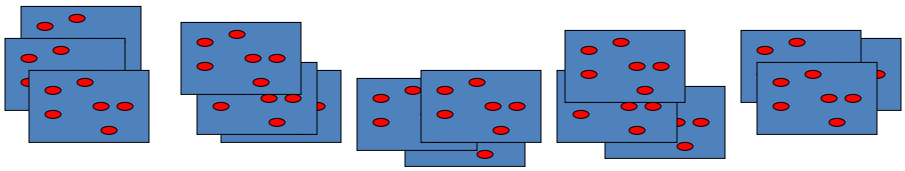
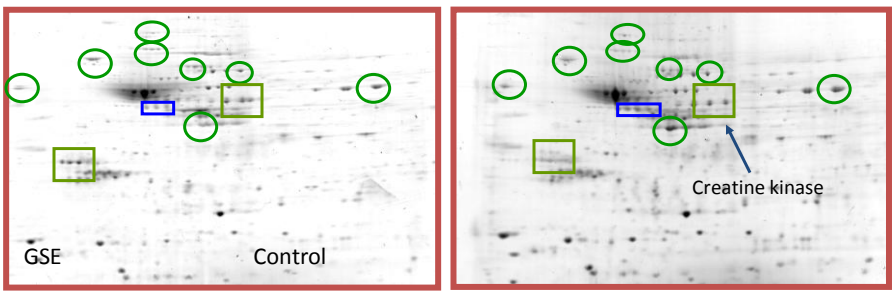


Image analysis indicated rat brain proteins were affected by GSE.



○ Different in intensity

□ Different in horizontal position

Database of protein differences in GSE vs CT brains

Protein Name	#matched pep	Accession #	MOWSE	Obs m.w.	Pred m.w.	Obs pI	Pred pI	Nature of change in GSE brains
Mitochondrial matrix proteinprecursorP60	10	P19227	*1.26E+04	64900	60956	5.6	5.9	+1.5
Creatine Kinase BB chain	12	P07335	*1.66E+05	45600	42712	5.45	5.3	+1.52 Translocation to Acidic pH
Actin	8	P10365	*2.18E+05	42000	41636	5.3	5.4	Less complex
GFAP	20	P47819	*9.67E+09	49000	49943	5.4	5.3	- 1.6
14-3-3 epsilon	10	P42655	*1.41E+09	31900	29174	4.49	4.6	- 2.1
Alpha Enolase	9	P04764	*6.64E+05	46000	46985	6.0	6.2	Less complex
Gamma Enolase	10	P07323	95	47000	47111	5.12	5.03	Less complex
RIKEN cDNA (NM 025994)	9	NP080270	169 95	26000 26000	25084 25084	5.0 5.1	5.0	-1.56
HSC-70	12	gi4103877	110	70321	42455	5.9	6.64	+1.63
HSC-71	16	gi123644	105	70386	71195	5.43	5.49	+1.91
Neurofilament L Triplet protein	14	gi13929098	120	61025	61298	4.61	4.63	+1.63
Neurofilament M triplet protein	19	gi8393823	153	95086	95591	4.75	4.76	+1.73
Vimentin	10	gi202368	93	53600	53641	5.09	5.06	-1.52

(Deshane et al., 2004. J. Agric. Food Chem.)

Initial conclusion: GSE is neuroprotective, since its effects on proteins are counter to the directions of change for the same proteins in disease.

Mitochondrial matrix protein precursor HSP-60 ↑
14-3-3 epsilon protein ↓
Heat shock cognate 70 ↑
Heat shock cognate 71 ↑
Neurofilament triplet protein L ↑
Neurofilament triplet protein M ↑
GFAP ↓
Vimentin ↓
Creatine kinase Brain isoform ↑
Gamma enolase
Alpha enolase
Actin
RIKEN cDNA NM 025944

GREEN: folding/ stress response
PURPLE : energy
BLUE: new
ORANGE: cytoskeleton

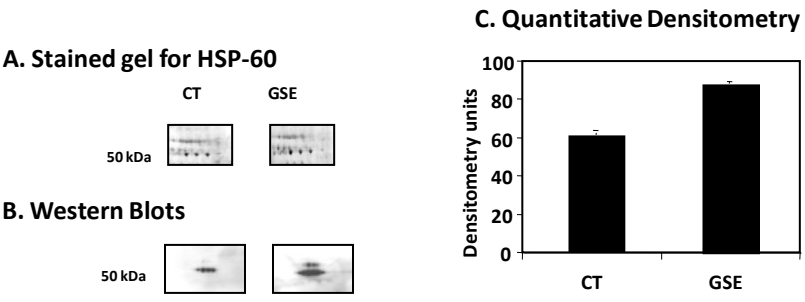
(Schonberger et al., 2001)

(Tilleman et al., 2002a)

(Tilleman et al., 2002b)

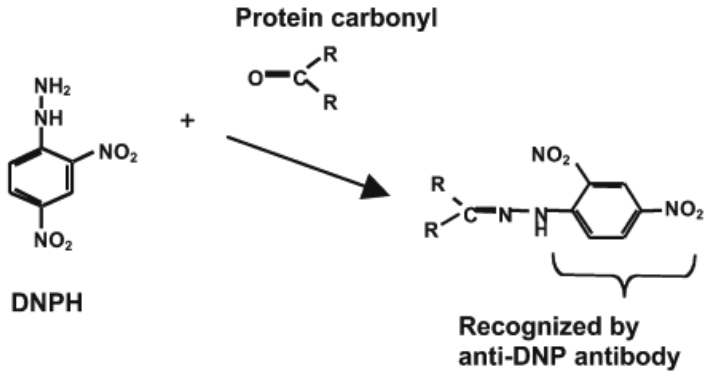
(Deshane et al., 2004. J. Agric. Food Chem.)

Western blot analysis corroborated 2D gel image analysis quantitation

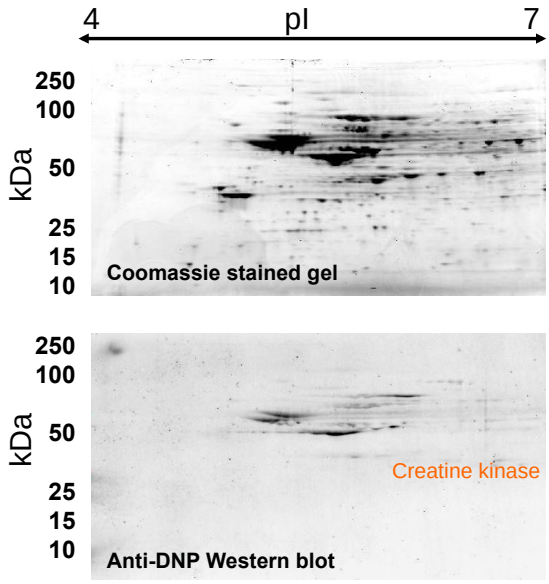


(Deshane et al., 2004. J. Agric. Food Chem.)

Use chemistry to determine whether GSE affects oxidations



Preliminary experiments indicated GSE affected oxidations of abundant proteins



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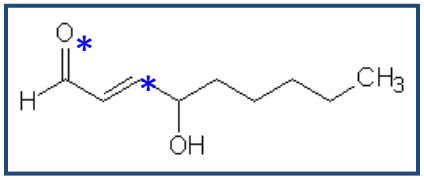
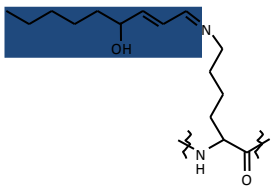
4-hydroxy-2-nonenal (4HNE)

A reactive aldehyde resulting from arachidonic acid

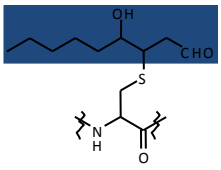


Reacts with K, C, and H



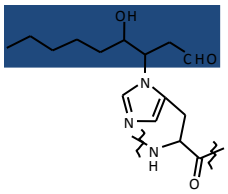
4HNE-Modified Lysine

Schiff Base Adduct



4HNE-Modified Cysteine

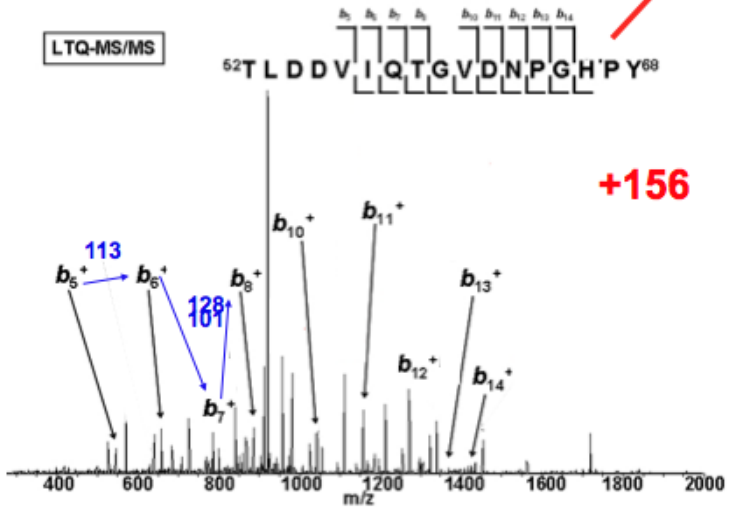
Michael Adduct



4HNE-Modified Histidine

Michael Adduct

MS/MS spectrum analysis confirmed a 4HNE Michael Adduct on H^{66}



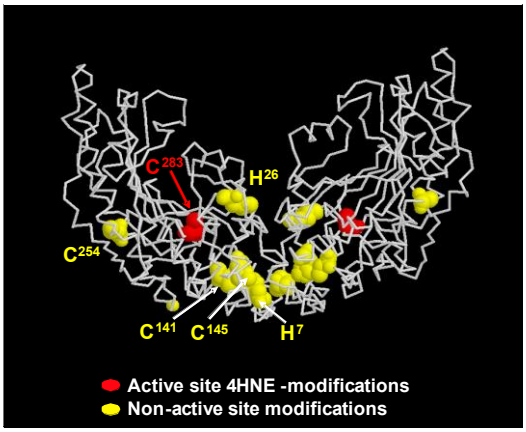
(Eliuk et al., *Chem. Res. Toxicol.*, 2007)

Modified Amino Acid	Concentration of 4HNE (_M)					
	5000	300	100	30	10	5
H^{7}	M,S	M,S	M,S	S		
H^{26}	M	M	M	M		
H^{29}	M	M				
K^{45}	M					
H^{66}	M	M				
K^{86}	M	M				
H^{97}	M	M	M			
K^{101}	M	M				
C^{141}	M	M	M	M		
C^{145}	M	M	M	M		
K^{177}	M					
H^{191}	M	M	M			
H^{219}	M,S	M,S	M			
H^{234}	M,S	M,S	S			
K^{247}	M,S					
C^{254}	M,S	M,S	M,S	M,S	M	M
H^{276}	M	M				
C^{283}	M	M	M	M	M	
H^{296}	M,S	M,S	S			
H^{305}	M	M				
K^{313}	M					
K^{358}	M					
K^{381}	M					

Sites of HNE-adducts on CKBB over a range of concentrations; RED indicates active-site residues

(Eliuk et al., *Chem. Res. Toxicol.*, 2007)

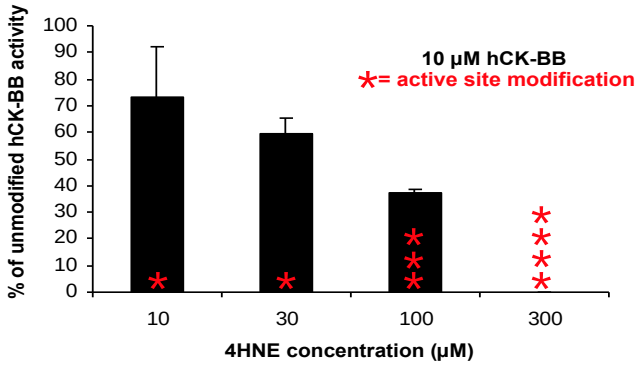
Crystal structure of CK-BB showing the HNE adducts at active-site and non-active-site residues detected at 30 μM 4HNE.



(crystal structure adapted from Eder et al., 1999)

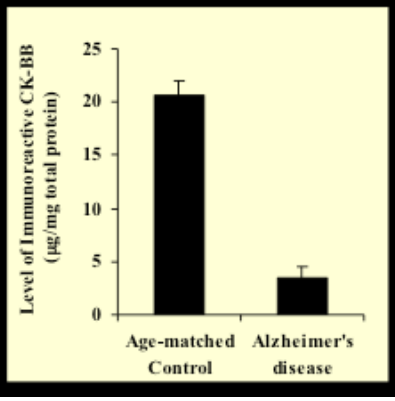
(Eliuk et al., *Chem. Res. Toxicol.*, 2007)

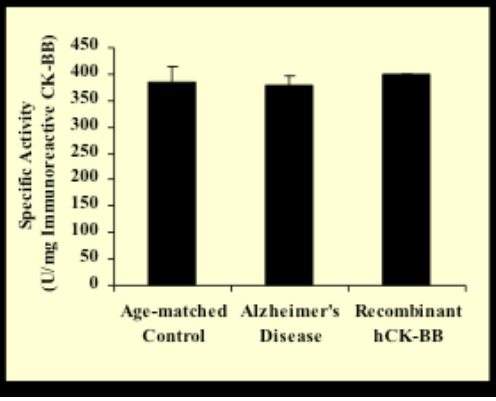
Reduction of CK-BB activity correlated with increased HNE-adducts formed at active-site residues



(Eliuk et al., *Chem. Res. Toxicol.*, 2007)

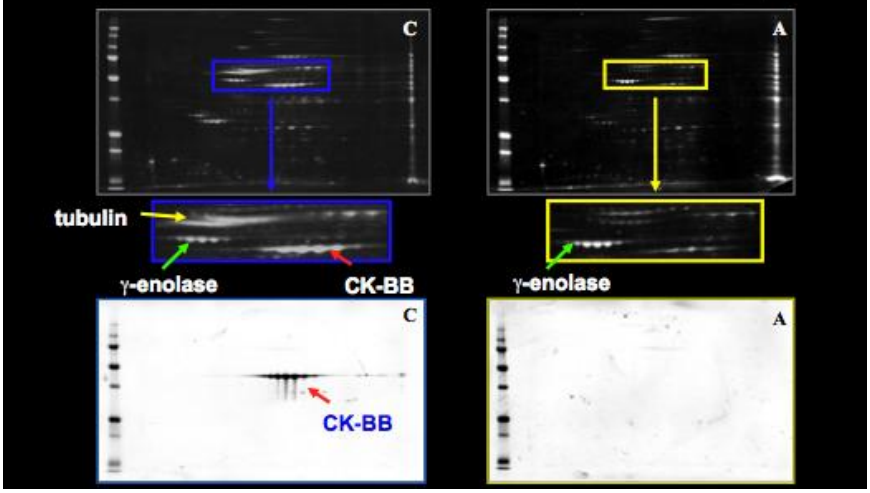
Specific Activity of CK-BB in AD or CT cytosol was not different



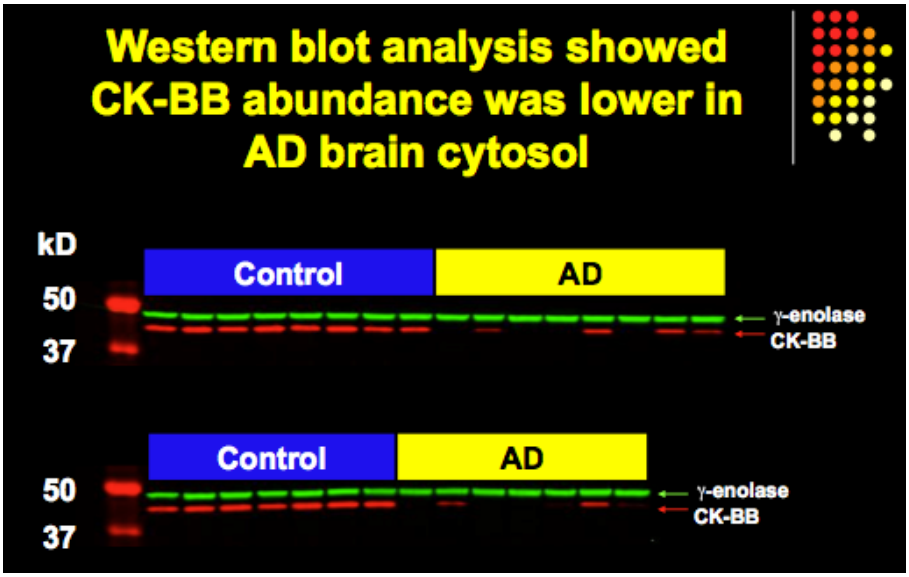


(Eliuk et al., *Chem. Res. Toxicol.*, 2007)

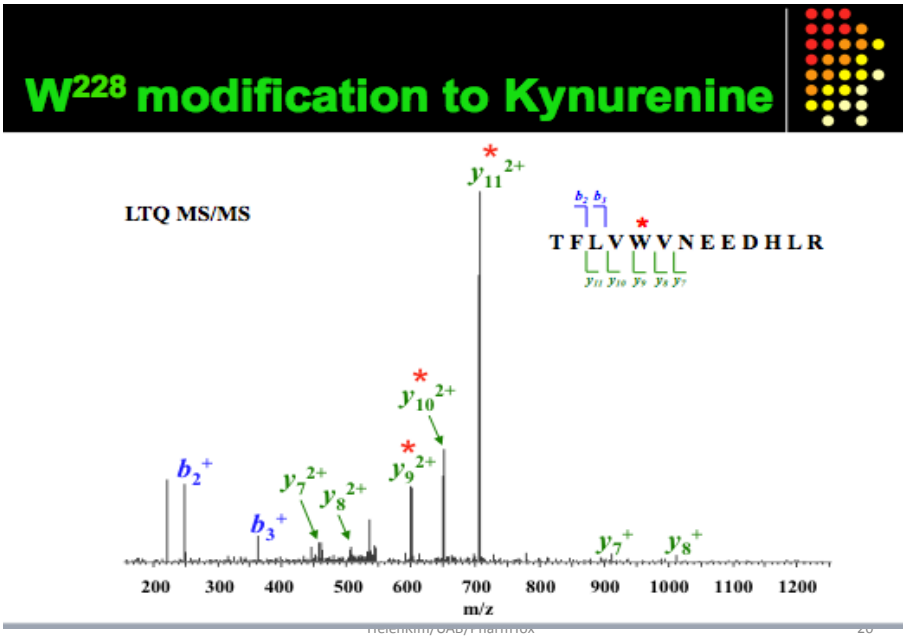
2D-gels showed decreased CK-BB in AD cytosol fractions



(Eliuk et al., *Chem. Res. Toxicol.*, 2007)

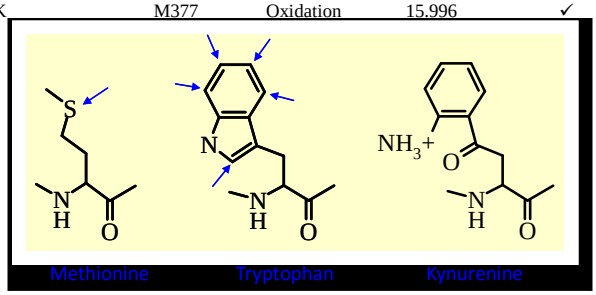


Modifications of CKBB in both AD and CT brain:



Modifications on CKBB detected by MS/MS analysis

Peptide Sequence	Modified Amino Acid	Modification	Mass Shift	AD	Age-matched Control
FPAEDEFPDLSAHNNHMAK	M30	Oxidation	15.998	✓	✓
FPAEDEFPDLSAHNNHMAK	M30	Di-oxidation	32.001	✓*	✓*
GIWHNDNK	W218	Oxidation	15.980	✓	✓
TFLVWVNEEDHLR	W228	Kynurenine	3.999	✓*	✓**
TFLVWVNEEDHLR	W228	Oxidation	16.005	✓	✓
TFLVWVNEEDHLR	W228	Di-oxidation	32.004	✓	✓
SKDYEFMWNPH	M272	Oxidation	15.997	✓	✓
LGFSEVELVQMVVDGVK	M352	Oxidation	15.990	✓	✓
LLIEMEQR	M363	Oxidation	15.995	✓	✓
LEQGQAIDDLMPAQK	M377	Oxidation	15.996	✓	✓



Structures courtesy of D. Stella

Conclusions regarding the grapeseed studies

- **GSE has pleiotropic effects in the brain:**
  - **gene expression/protein turnover;**
  - **protein oxidations;**
  - **These actions may be consistent with neuroprotection, since the majority are in the opposite direrction to changes affecting these proteins in AD or models of dementia.**
  - **These were the first studies to identify specific proteins affected by dietary intake of a complex botanical mixture.**

Conclusions from the studies with CKBB

- Incubation with HNE stoichiometrically inhibited hCKBB activity.
  - this was correlated with increased HNE adducts on CKBB, revealed by FT-ICR-MS.
- At the lowest activity, all four active-site residues of CKBB were HNE-modified.

- Thus, a combination of state of the art mass spectrometry and conventional biochemistry was optimal in determining the role of HNE adducts on CKBB function.

More conclusions from studies of CKBB

- In AD brain, CKBB was not detectably modified with HNE;
- Rather, oxidative modifications on CKBB were similar between AD and CT brains.
  - These non-differences correlated with a lack of difference in specific activity of CKBB between AD and CT.
- HOWEVER, a small % of CKBB (cytosolic isoform) was detected in a particulate form in both AD and CT, but more so in AD; this DID have lower specific activity. THIS CKBB may be interestingly modified.