31 mai & 1 juin 2016

adebiotech stress ox

Modulation du stress oxydant

Nouveaux concepts pour de nouvelles applications

BIOCITECH, CITÉ DES ENTREPRISES DE SANTÉ ET DE BIOTECHNOLOGIES, ROMAINVILLE

Detection, quantification and identification of oxidized proteins in biological systems

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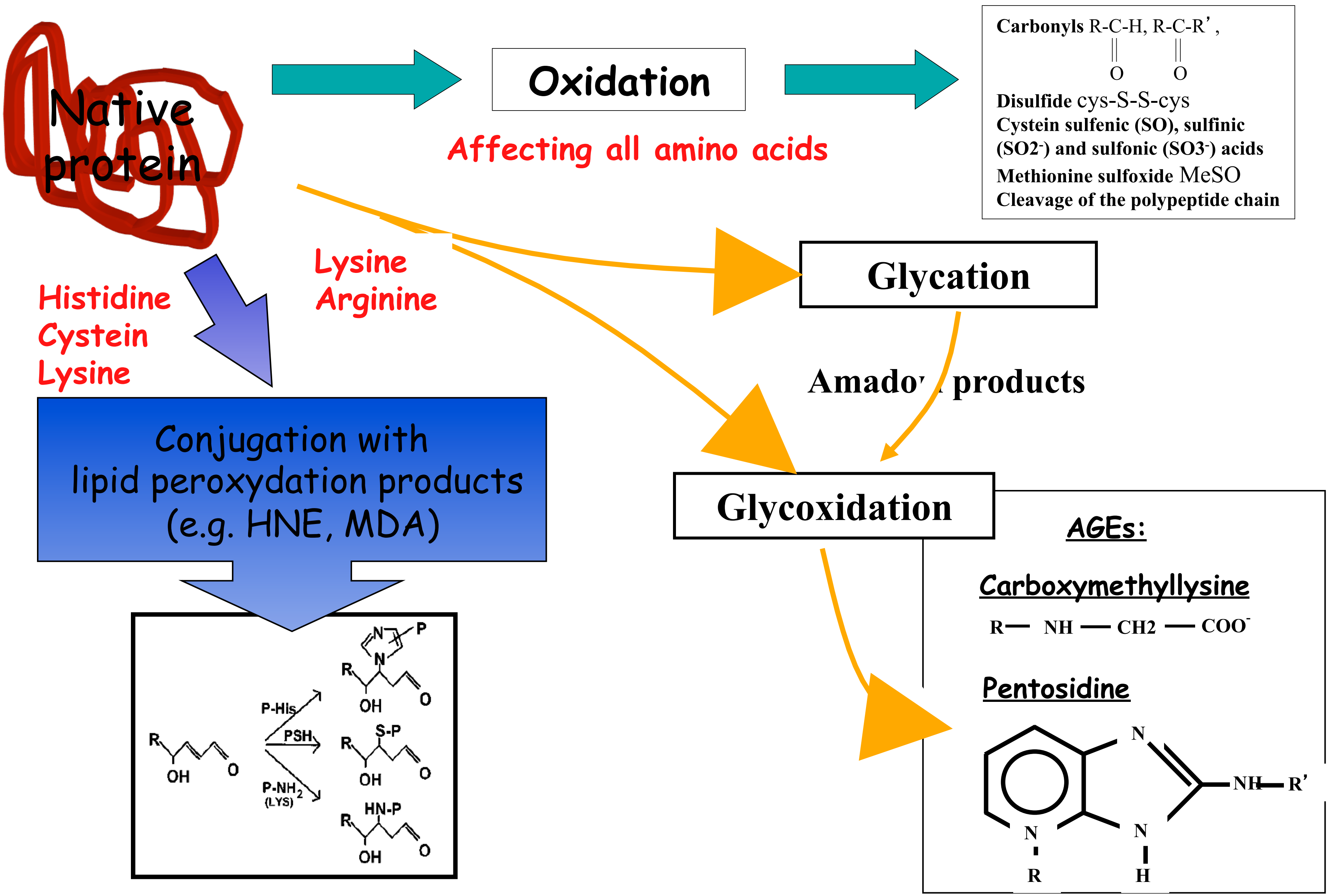
Protein modifications by oxidation and related pathways



Impact of protein carbonylation

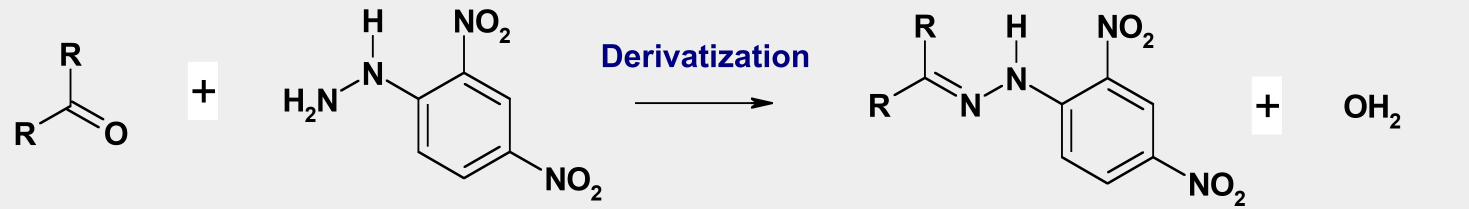
1. Biochemical activity
   (loss of function)

2. Protein misfolding and aggregate formation
   (gain of toxic function)

3. Cellular targeting
   (protein localization)

4. Cellular metabolism and signaling
   (protein-protein interaction, cascade
   amplification)

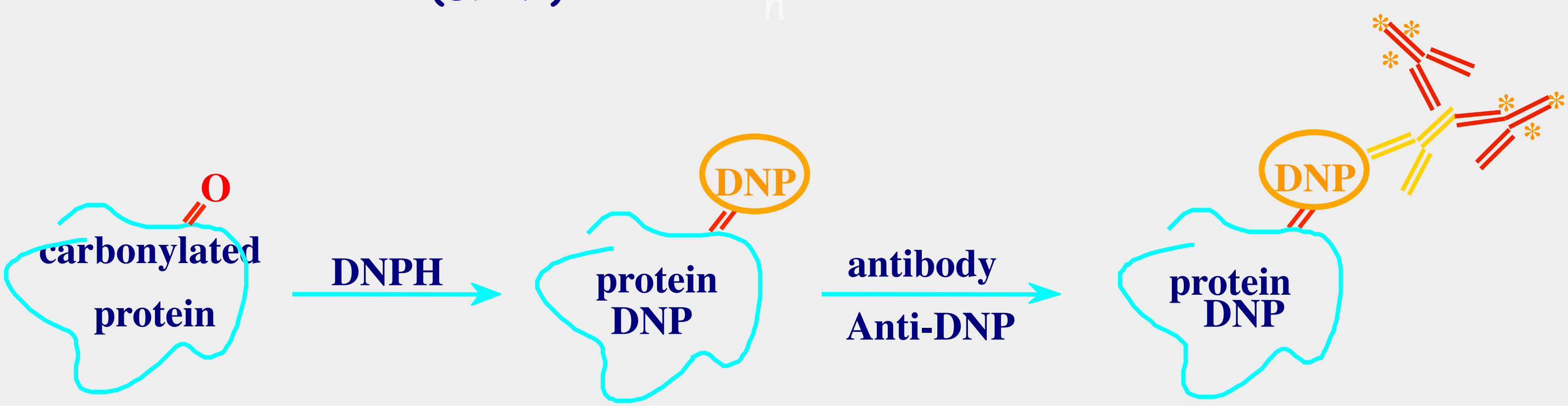
Immunodetection of protein carbonyl groups



Carbonyl group

Dinitrophenyl-hydrazine (DNPH)

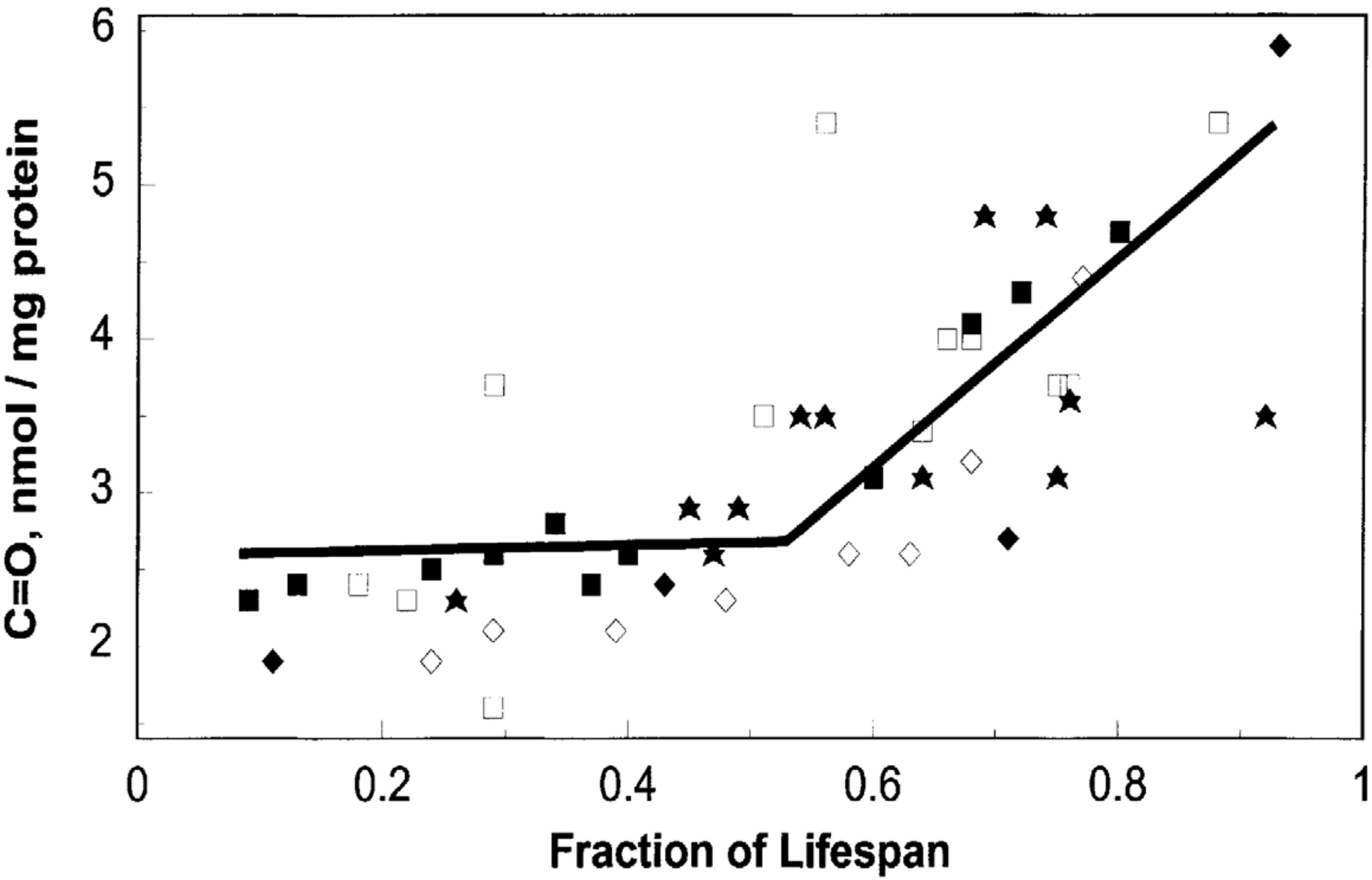
Dinitrophenyl-hydrazone



Western blot anti-DNP

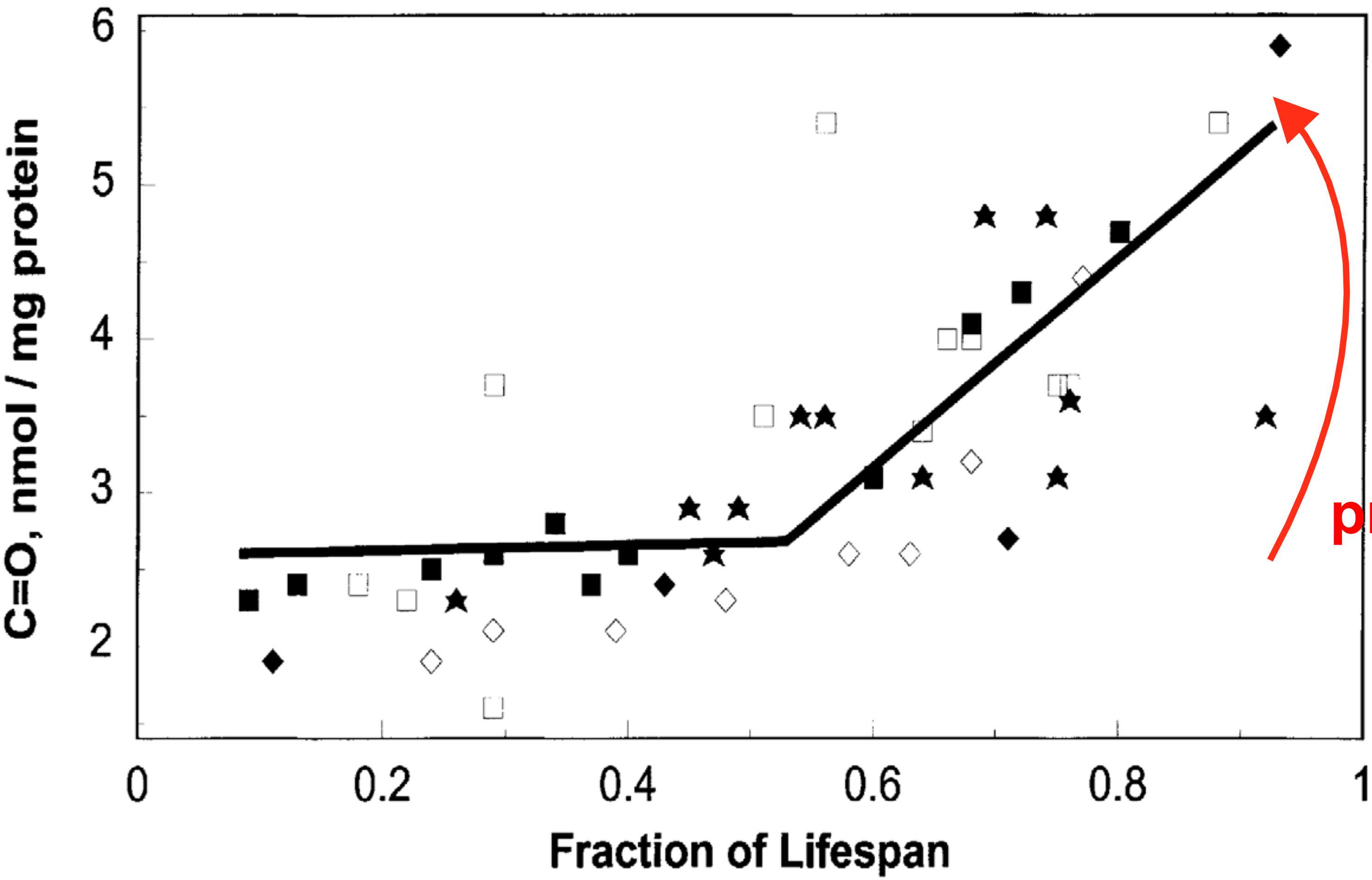
Shacter *et al*. (1994) *Free Radic. Biol. Med.* **17**

Age-related accumulation of oxidized proteins



From: Levine and Stadtman, 2001, Exp. Gerontol., 36:1495-1502
(human dermal fibroblasts, human lens, human brain, rat liver, whole fly)

Age-related accumulation of oxidized proteins



2 fold increase !

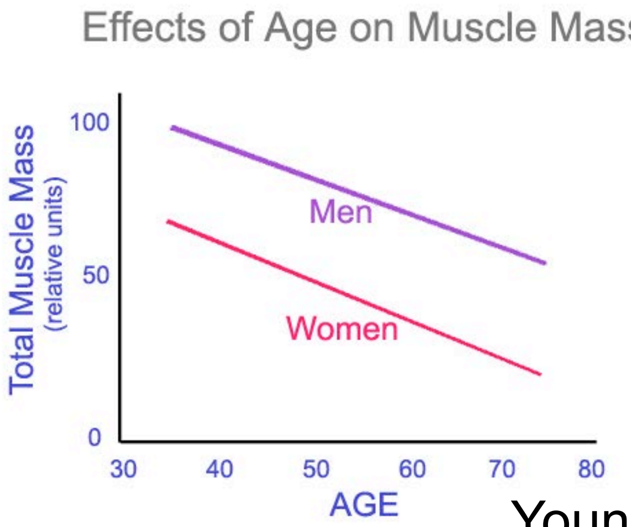
Specific protein targets for oxidative damage !!

From: Levine and Stadtman, 2001, Exp. Gerontol., 36:1495-1502
(human dermal fibroblasts, human lens, human brain, rat liver, whole fly)

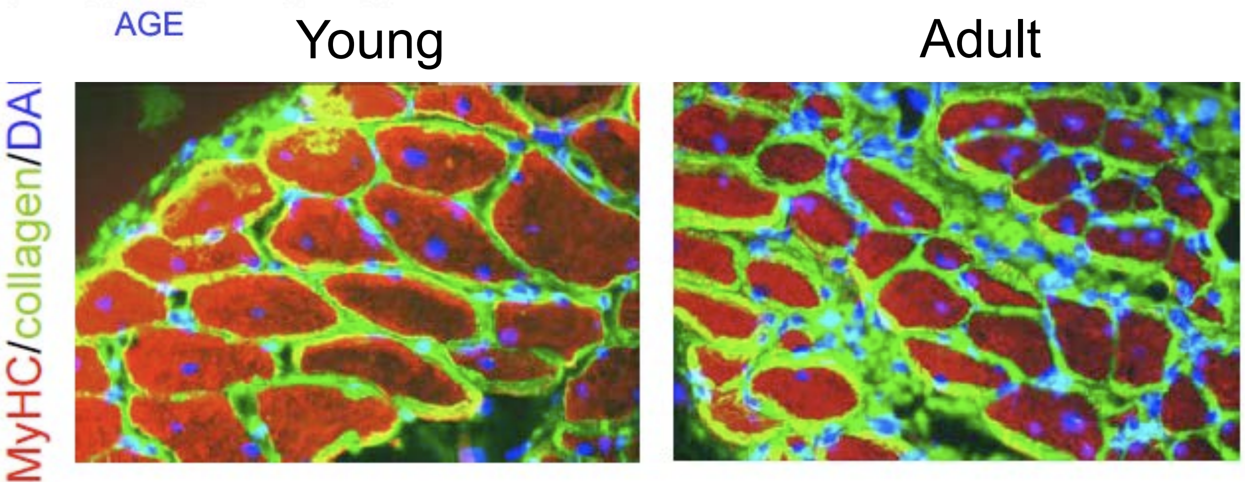
Understanding and combating age-related muscle weakness
FP7 - IP 223576 (2009-2014)

Leader of WP6 on « Post-translational modifications of proteins and protein turnover »

Skeletal Muscle Ageing

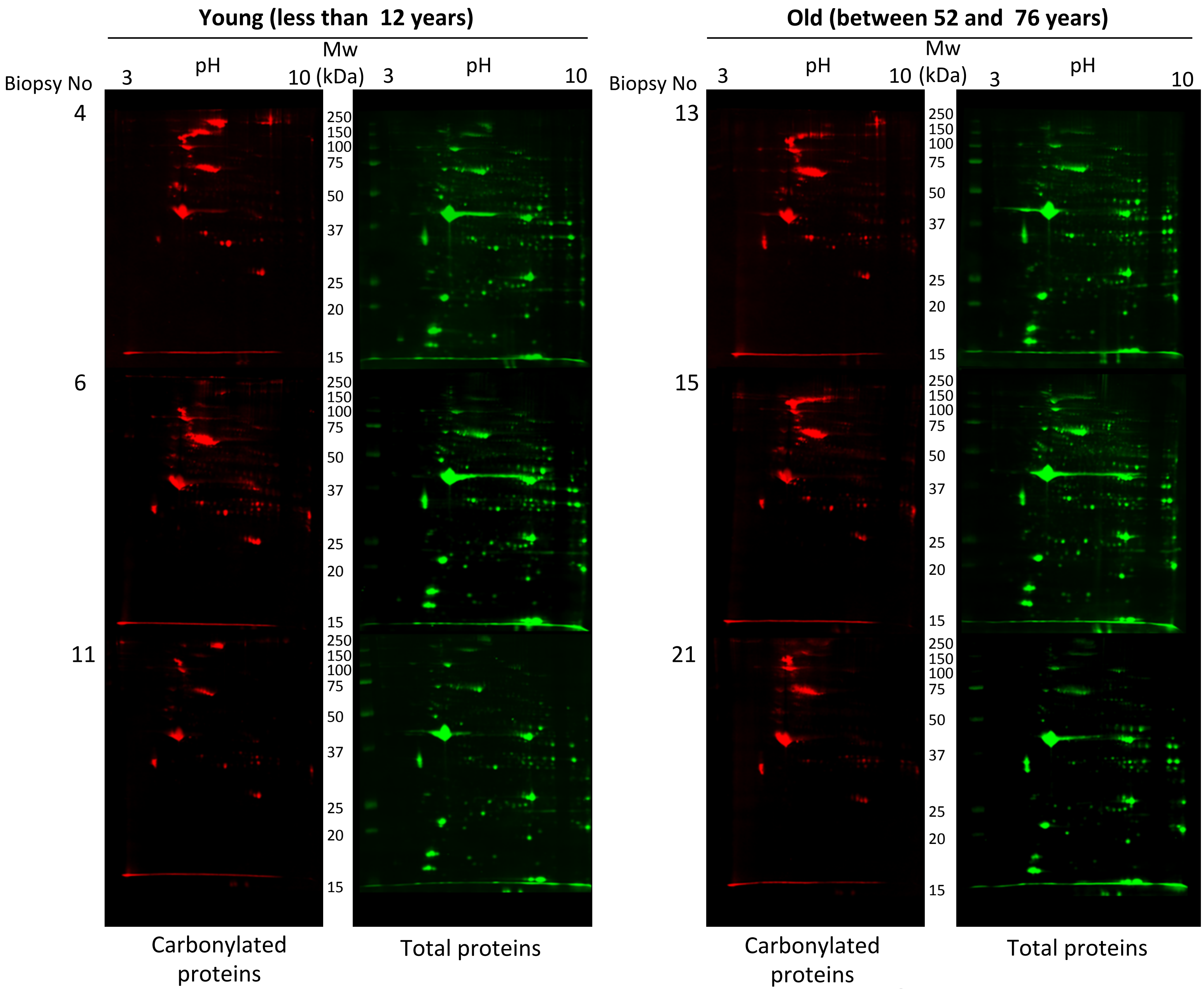


- ✓ Results in a progressive loss of mobility that decrease the quality of life
- ✓ Increasing muscle weakness is a major component of muscle ageing



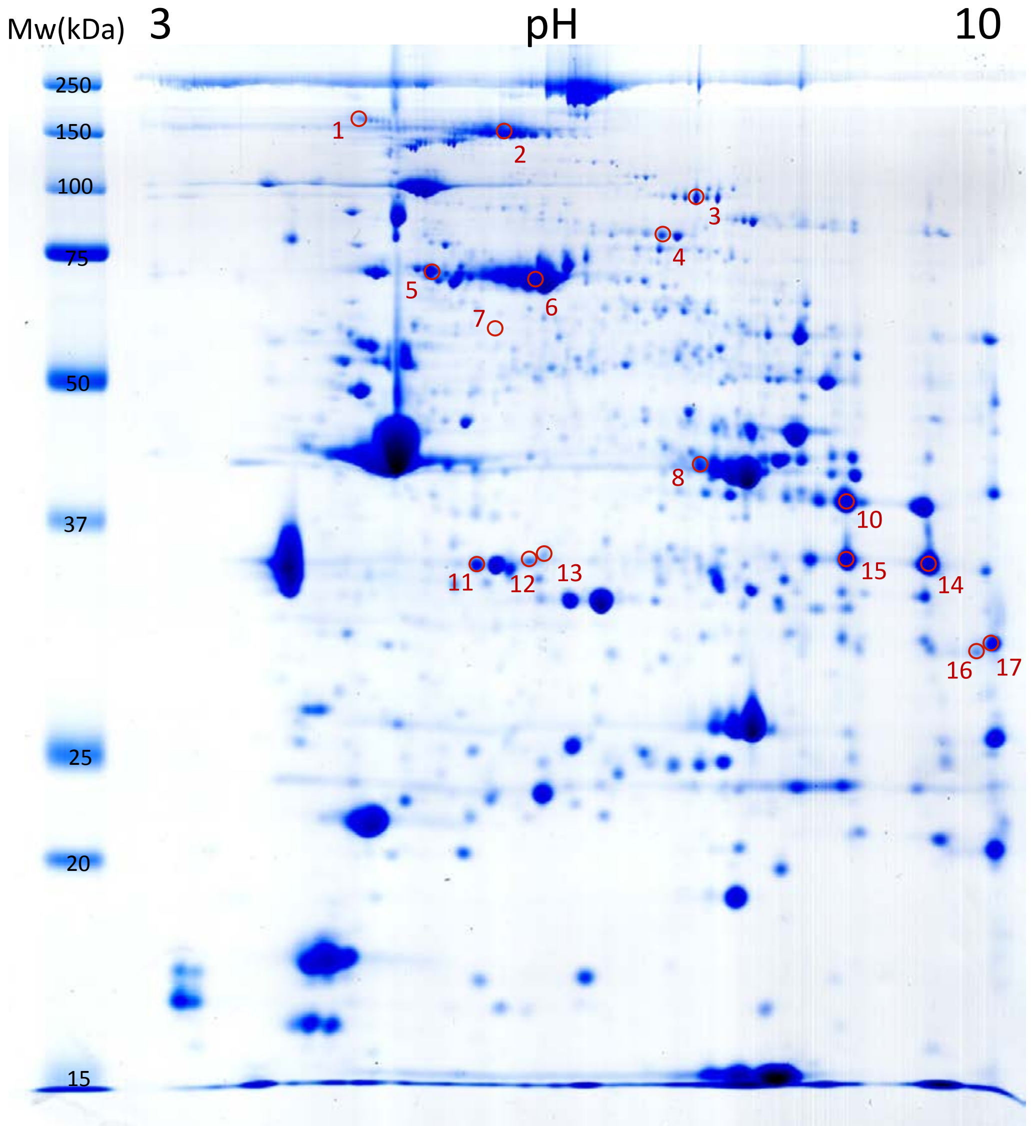
Brack AS et al. Science. (2007)

2D Oxi-proteome analysis of young and old human skeletal muscle



Lourenço dos Santos et al. 2015, Redox Biology

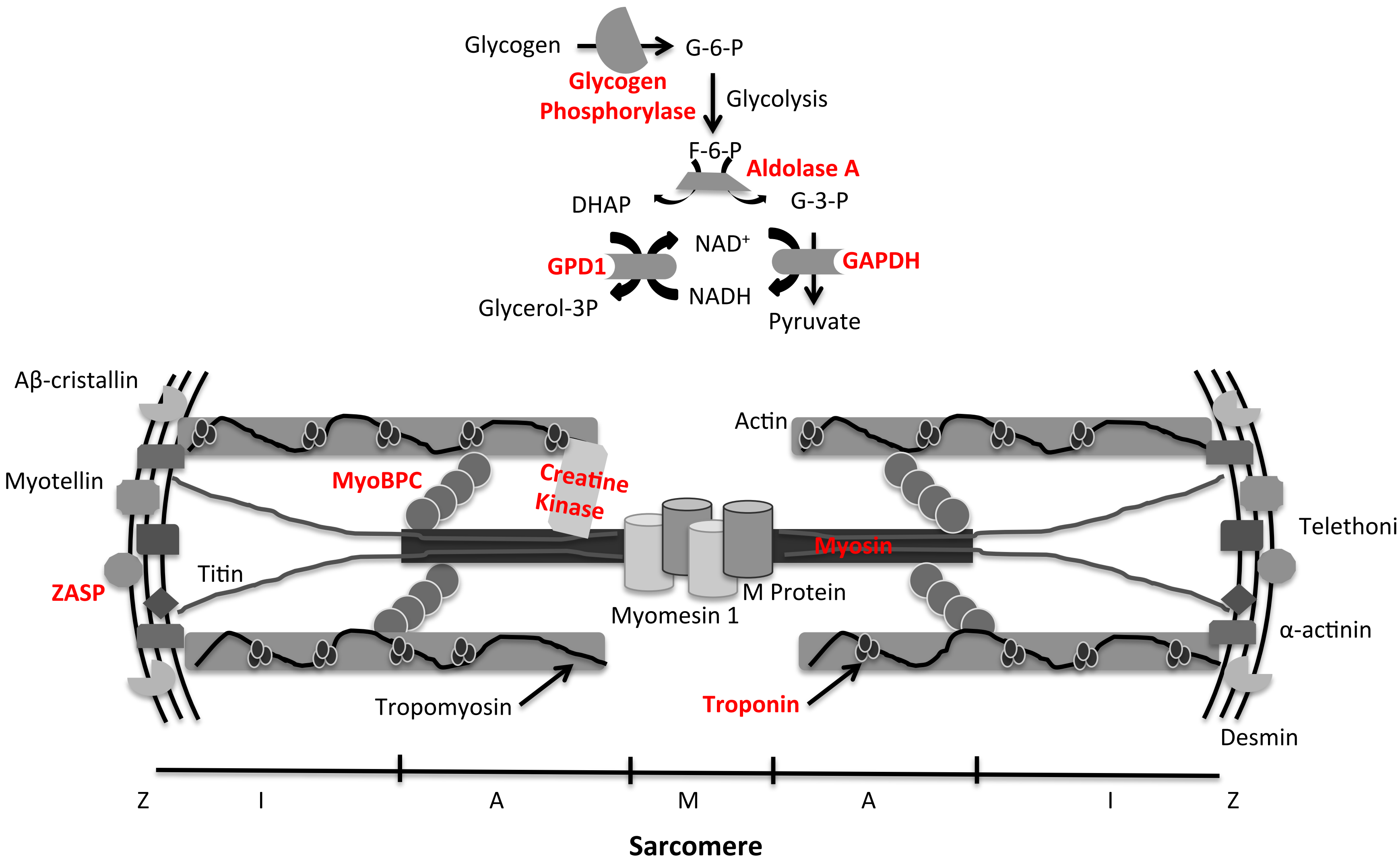
Coomassie blue staining of a representative 2D gel of *Rectus Abdominis* muscle: spots picked for MS identification



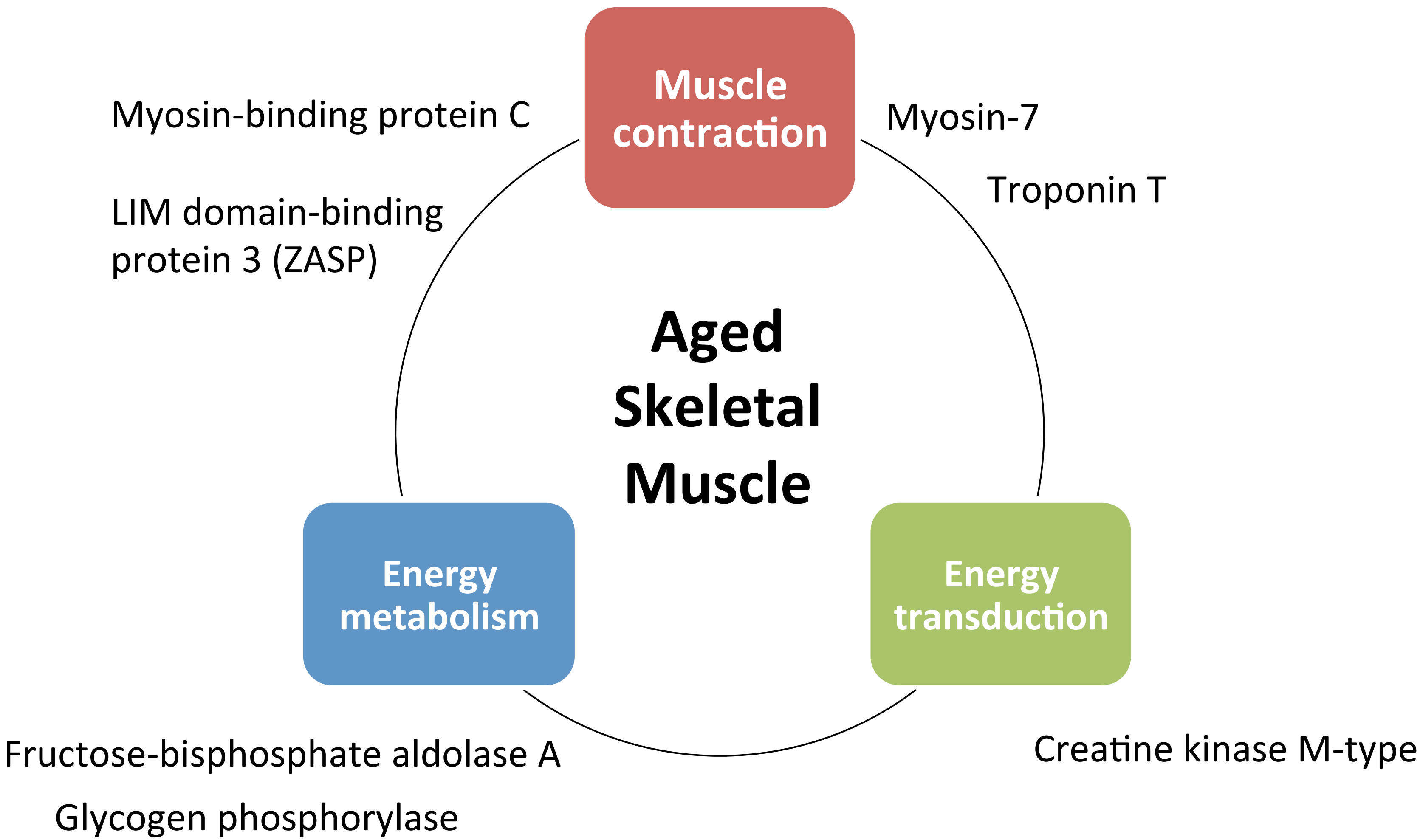
Identification of increasingly carbonylated proteins in old muscle

	Protein name	Swiss-Prot accession no	Mascot score	Sequence coverage (%)	Theoretical mass (kDa)	Theoretical PI	RMI ratio
Ubiquitous Proteins	Collagen alpha-1(VI) chain	CO6A1_HUMAN	205	7	108	5.3	4.34
	Heat shock cognate 71 kDa protein	HSP7C_HUMAN	256	15	71	5.4	1.63
	Glycerol-3-phosphate dehydrogenase [NAD+], cytoplasmic (GPD1)	GPDA_HUMAN	214	22	38	5.8	1.52
	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	G3P_HUMAN	243	12	36	8.6	1.67
	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	G3P_HUMAN	424	25	36	8.6	1.68
	Voltage-dependent anion-selective channel protein 1	VDAC1_HUMAN	685	39	31	8.6	1.88
Muscle Specific Proteins	Myosin-binding protein C, slow-type (MyBPC)	MYPC1_HUMAN	119	3	128	5.8	1.54
	Glycogen phosphorylase, muscle form	PYGM_HUMAN	882	22	97	6.6	1.67
	Myosin-7	MYH7_HUMAN	63	-	223	5.6	3.12
	Creatine kinase M-type	KCRM_HUMAN	206	9	43	6.8	1.44
	Creatine kinase M-type	KCRM_HUMAN	416	20	43	6.8	1.54
	Fructose-bisphosphate aldolase A	ALDOA_HUMAN	611	31	39	8.3	1.65
	Troponin T, slow skeletal muscle	TNNT1_HUMAN	512	22	33	5.9	1.87
	Troponin T, slow skeletal muscle	TNNT1_HUMAN	244	22	33	5.9	1.42
	LIM domain-binding protein 3 (ZASP)	LDB3_HUMAN	206	8	77	8.5	1.70

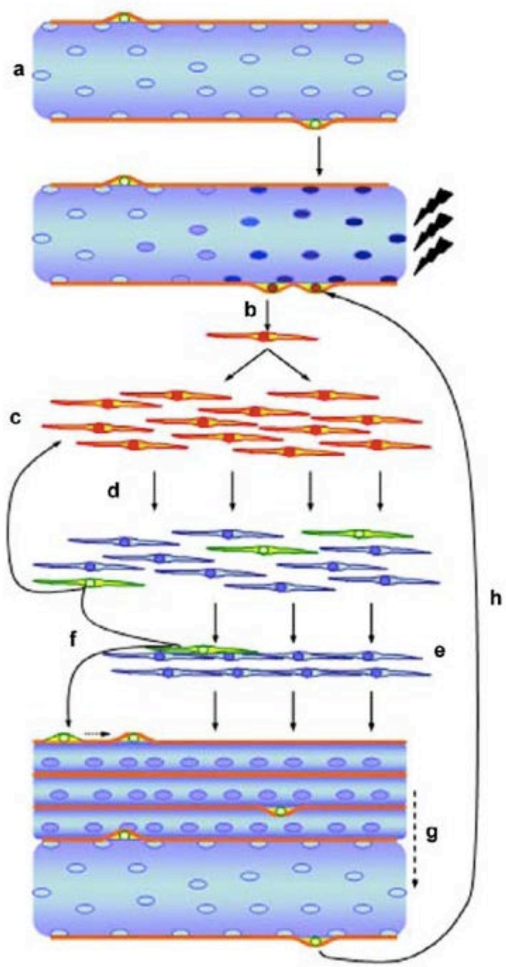
Aged Rectus Abdominis Muscle Oxi-Proteome



Functional grouping of skeletal muscle proteins increasingly oxidized with age

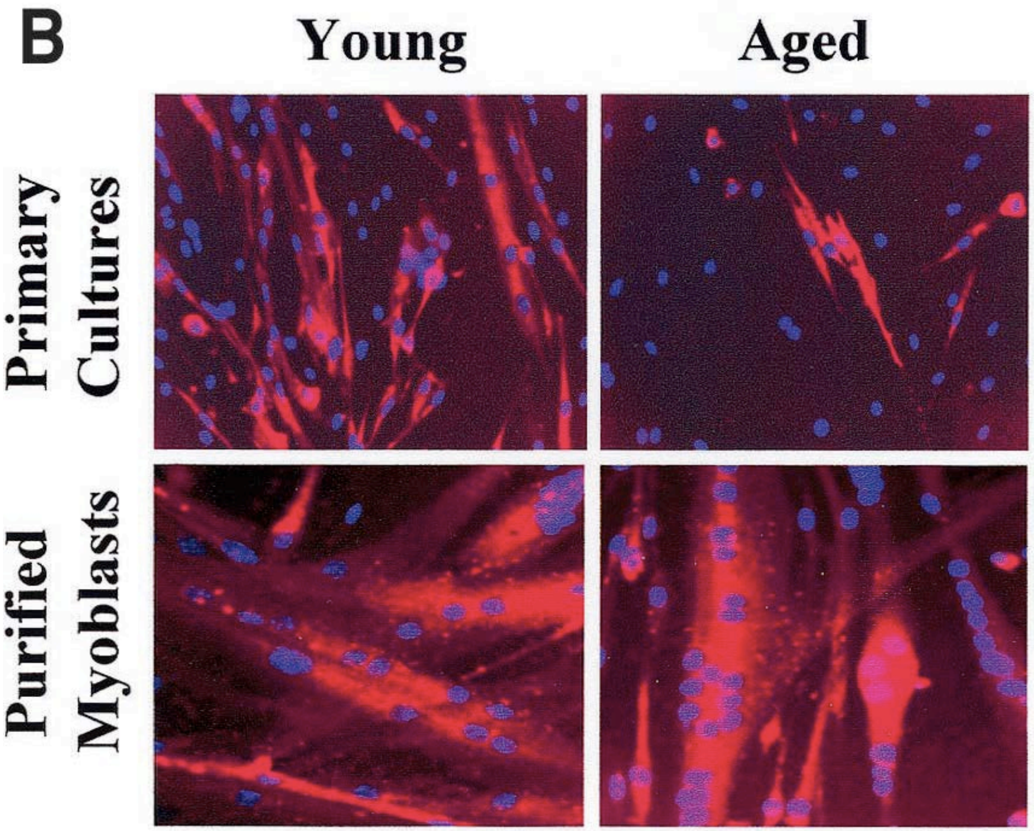


Human muscle progenitor or satellite cells are key players in skeletal muscle regeneration



Collins A et al. Cell (2005)

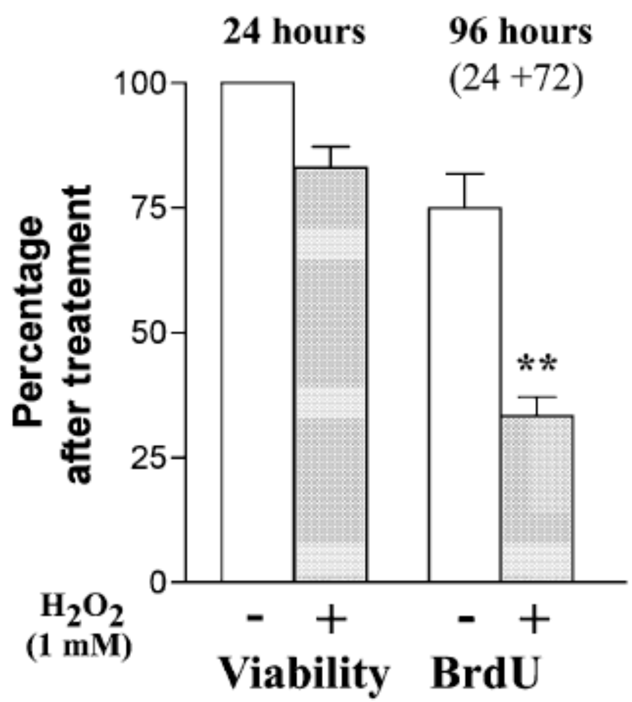
Reduced ability to proliferate and to differentiate into new myofibers during aging



Conboy I et al. Science. (2005)

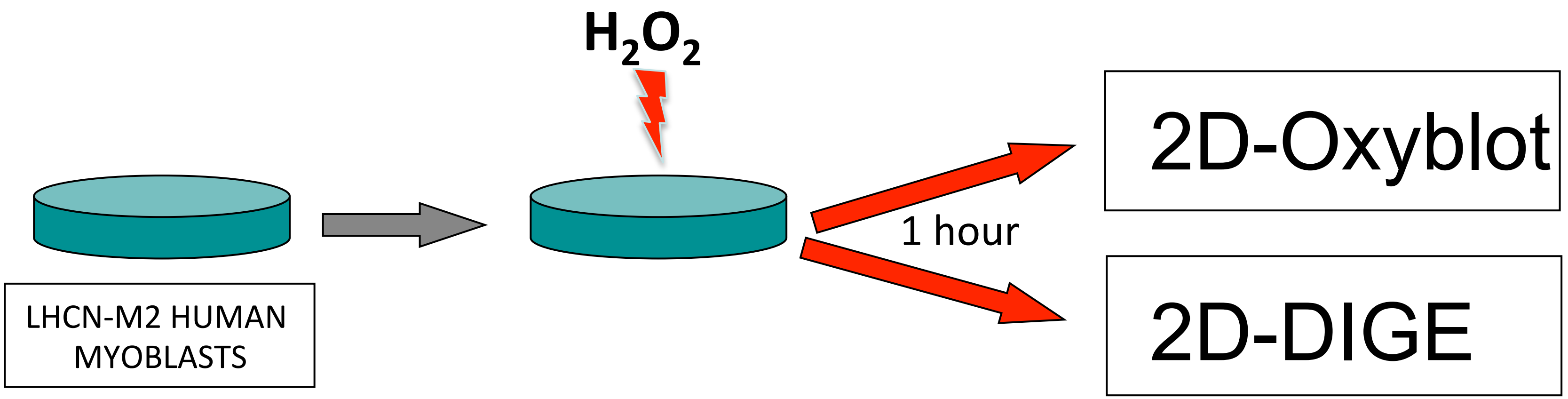
Deleterious effects of oxidative stress on human myoblasts proliferation

⬇ Proliferative Capacity



Renault et al. Exp Geront . (2002)

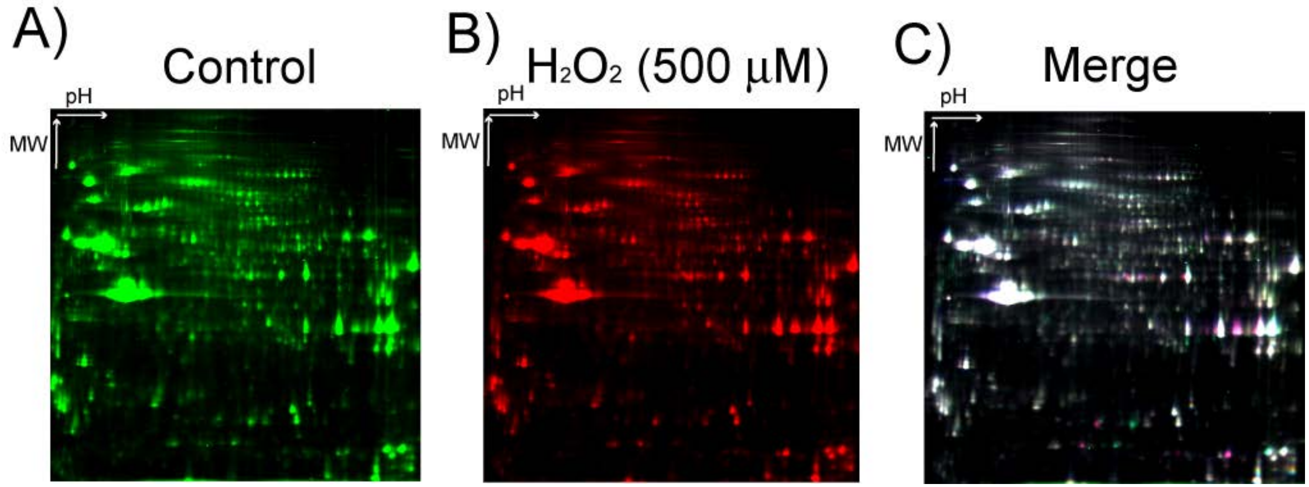
Oxidized protein targets in myoblasts upon acute oxidative stress



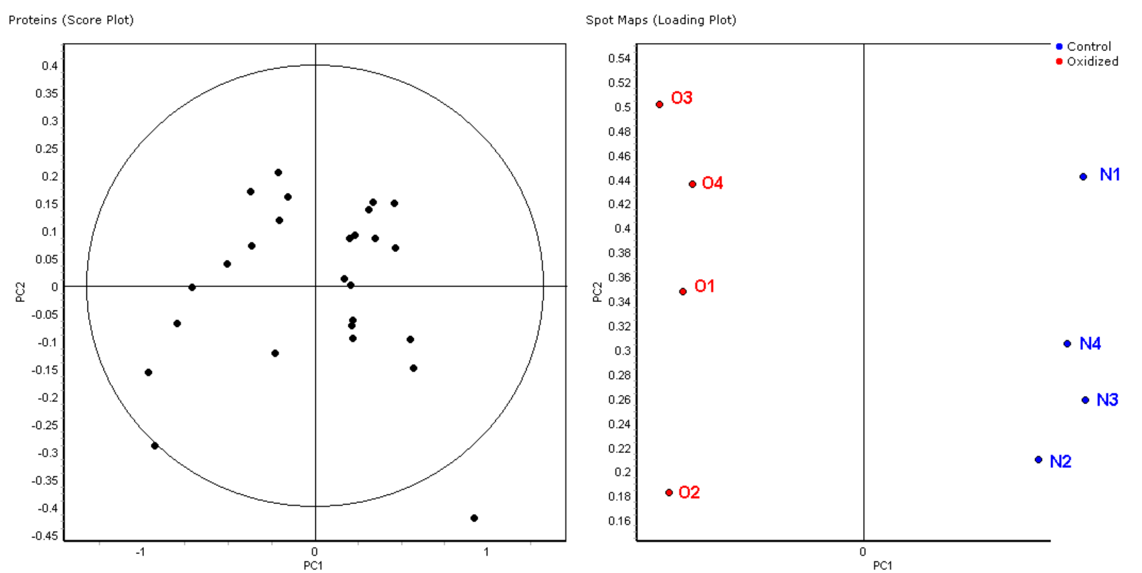
$[H_2O_2]$ (mM)		0	0.5	1
Cell viability (%)		100	92.6±5.2	52.1±9.6*
Protein carbonyls increase (%)		-	42.6±7.6*	43,7±5.8*
Proteasome activity (% of control)	CT-L	100%±7%	66%±9%*	77%±9%*
	T-L	100%±9%	76%±13%	32,5%±65%*
	Casp-L	100%±6%	59%±5%*	93%±7,5%

Baraibar et al., 2011, Free Radic Biol Med

Protein expression profile of human myoblasts upon oxidative stress



PCA POI - Pick List



Identification of responsive proteins upon oxidative stress

	Identified Protein	Swiss-Prot accesion number	Fold change	Score Mascot	Sequence coverage (%)	Sequenced peptides	No. Of matched peptides	Theoretic Mass (Da)	Theoretic PI
Up-regulated proteins									
1	Peroxiredoxin-1	Q06830	4.64	472	46	13	4	22096	8,27
2	Peroxiredoxin-6	Q06830	4.06	345	24	10	4	22096	8,27
3	Peroxiredoxin-6	P30041	4.05	310	34	12	3	25019	6,02
4	Protein DJ-1	Q99497	3.54	128	39	-	-	19878	6,33
5	Glyceraldehyde-3-phosphate dehydrognase	P04406	2.3	462	43	17	5	36030	8,58
7	Alpha-enolase	P06733	1.85	629	36	22	8	47139	6,99
8	Glyceraldehyde-3-phosphate dehydrogenase	P04406	1.46	793	44	27	11	36030	8,58
9	Caldesmon	Q05682	1.44	275	23	21	6	93194	5,63
10	Glyceraldehyde-3-phosphate dehydrogenase	P04406	1.41	266	31	13	4	36030	8,58
11	Adenylyl cyclase associated protein 1	Q01518	1.34	118	24	12	4	51869	8,26
Down-regulated proteins									
12	Proten transport Sec23A	Q15436	-1.31	231	11	12	5	86105	8,26
13	Disulfide-isomerase A3	P30101	-1.34	798	27	23	10	56747	5,61
14	Heat shock protein β-1	P04792	-1.4	1340	36	35	18	72288	5,01
15	78 kDa glucose-regulated protein	P11021	-1.41	742	31	25	13	60864	5,43
16	Prolyl 4-hydroxylase	O15460	-1.42	1060	65	43	13	47139	6,99
17	Alpha-enolase	P06733	-1.44	342	28	17	6	61011	5,7
18	Prolyl 4-hydroxylase	P13674	-1.47	231	11	12	5	86105	8,26
19	ATP-citrate synthase	P53396	-1.6	123	13	16	2	120762	6,95
21	ATP-citrate synthase	P53396	-1.71	167	17	21	2	120762	6,95
22	ATP-citrate synthase	P53396	-2.03	95	11	15	2	120762	6,95
23	Glyceraldehyde-3-phosphate déhydrogénase	P04406	-2.09	790	46	23	8	36030	8,58
24	Péroxiredoxine-1	Q06830	-2.54	425	43	11	3	22096	8,27
25	Proteasome subunit ß3	P49720	-2.55	332	33	14	5	22933	6,13
26	Peroxiredoxin-6	P30041	-4.65	140	32	9	1	25019	6,02

Antioxidant response | Energy Metabolism | Protein Maturation | Protein Degradation

Identification of carbonylated proteins upon oxidative stress

Spot	Identified Protein	Swiss Prot accession number	Mascot Score	Sequence Coverage (%)	Seqenced Peptides	Matched paptides	Theoretic Mass (Da)	Theo retic pI	Ratio RMI
1	Elongation Factor 2	P13639	486	19	28	12	95207	6,42	2,5 ± 0,2
2	Elongation Factor 2	P13639	734	24	29	14	95207	6,42	1,8 ± 0,2
3	Elongation Factor 2	P13639	605	24	25	10	95207	6,42	7,2 ± 2,5
4	Elongation Factor 2	P13639	754	23	29	13	95207	6,42	h
5	Programmed cell death 6-interacting protein	Q8WUM4	513	31	29	10	95963	6,14	2,3 ± 1
6	Programmed cell death 6-interacting protein	Q8WUM4	361	29	30	6	95963	6,14	1,9 ± 0,1
7	Apha-glucosidase neutre AB	Q14697	675	33	39	15	103975	5,58	5,6 ± 1,5
8	Apha-glucosidase neutre AB	Q14697	675	33	39	15	103975	5,58	h
9	Gelsoline	P06396	979	29	26	11	82959	5,72	1,9 ± 0,6
10	Gelsoline	P06396	502	28	21	7	82959	5,72	1,7 ± 0,2
11	Gelsoline	P06396	185	17	14	5	82959	5,72	1,64 ± 0,1
12	Prolyl 4-hydrolase	O15460	723	37	28	11	58566	5,43	1,34 ± 0,01
13	Prolyl 4-hydrolase	O15460	723	37	28	11	58566	5,43	1,9 ± 0,1
14	Protein disulfide-isomerase A3	P30101	1030	42	35	12	54265	5,61	1,3 ± 0,1
15	Alpha-enolase	P06733	954	51	-	-	47037	6,99	1,6 ± 0,04
16	Alpha-enolase	P06733	1150	42	26	9	47037	6,99	1,7 ± 0,4
17	Protein Lupus La	P05455	135	15	11	9	46837	6,7	h
18	Proteasome subunit 10B 26S	P62333	526	24	17	8	44041	7,25	3,2 ± 0,3
19	Annexine A2	P07355	1410	46	30	15	38472	7,56	3,1 ± 0,8
20	Annexine A2	P07355	1510	56	32	19	38472	7,56	1,4 ± 0,12
21	Glyceraldehyde-3-phosphate dehydrogenase	P04406	307	32	9	3	35922	8,58	3,3 ± 0,8

Protein Synthesis

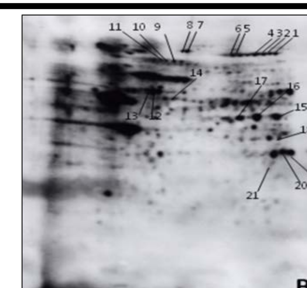
Protein Transport

Protein Maturation

Protein Degradation

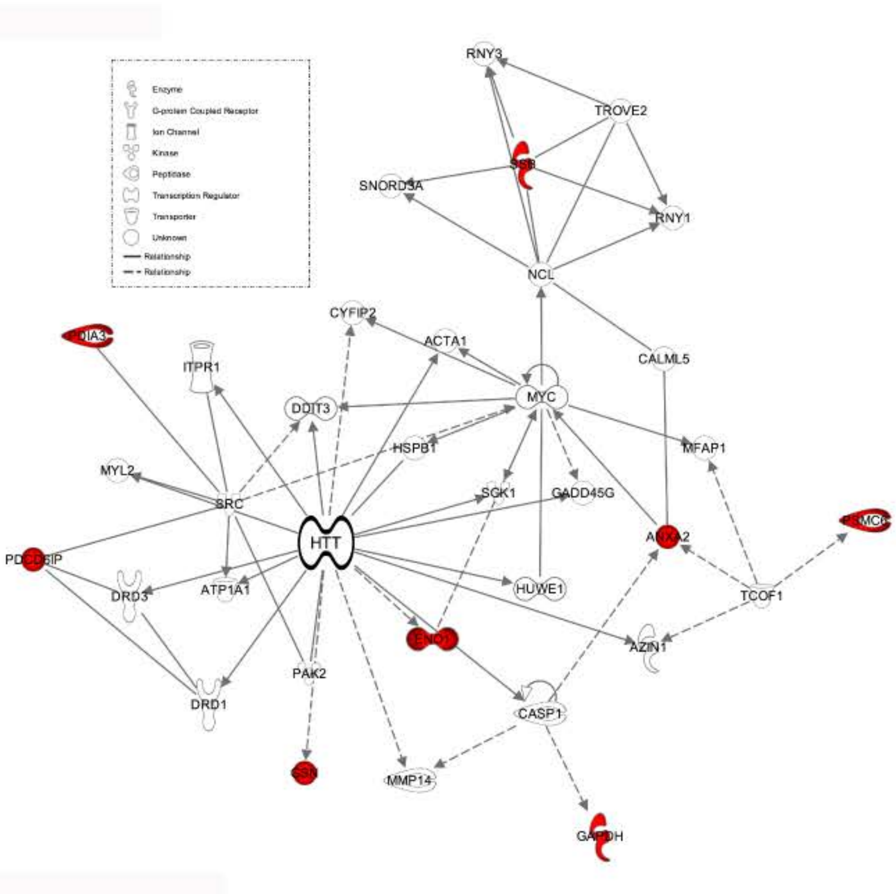
Cytoskeletal assembly

Energy Metabolism

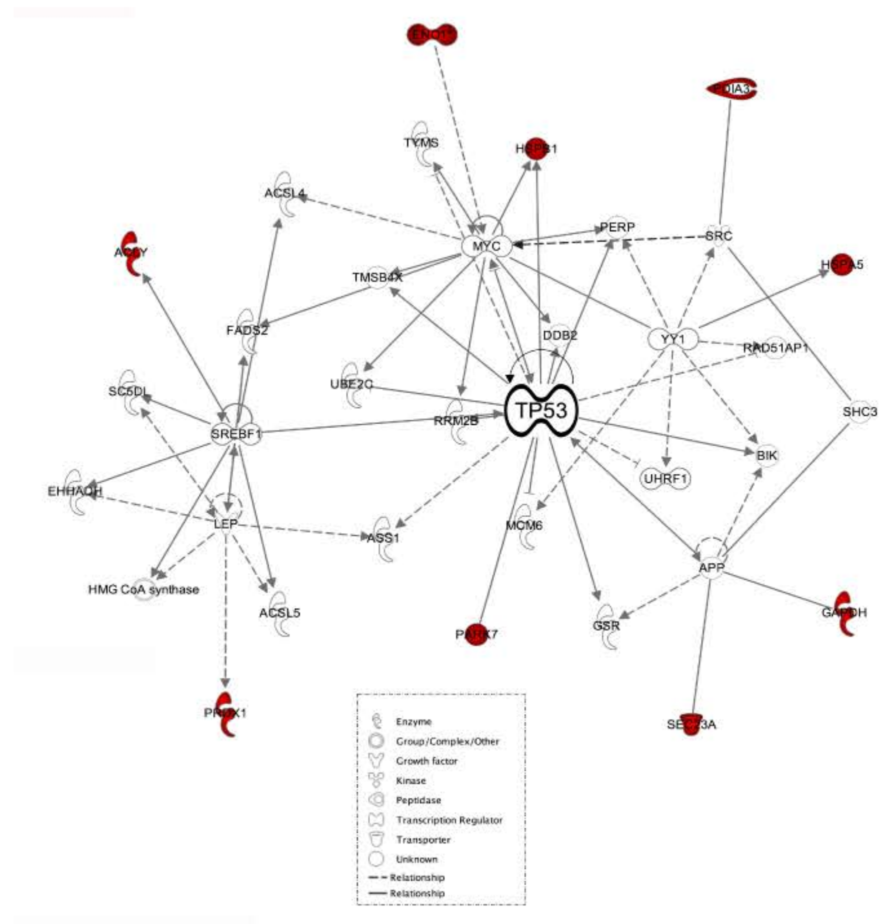


Cellular Pathway Analysis (Ingenuity™ Software)

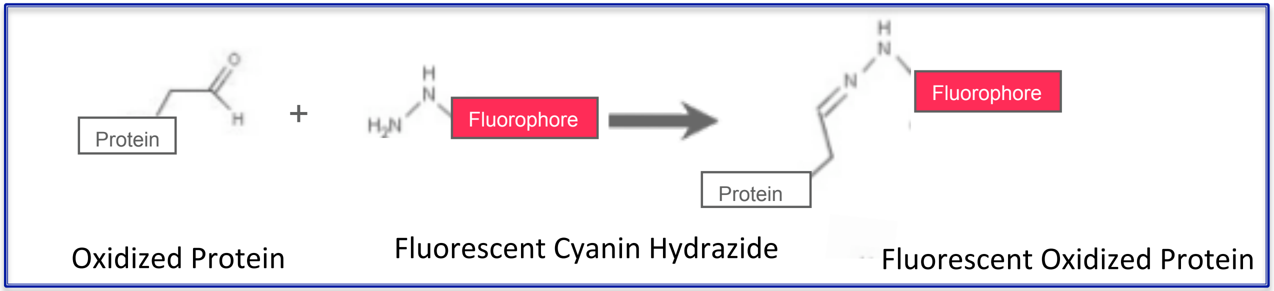
Carbonylated Proteins



Differentially Expressed Proteins



A novel approach for detecting and quantifying of carbonylated proteins: THE OXI-DIGE TECHNOLOGY

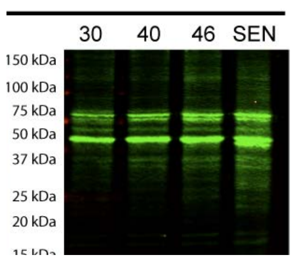


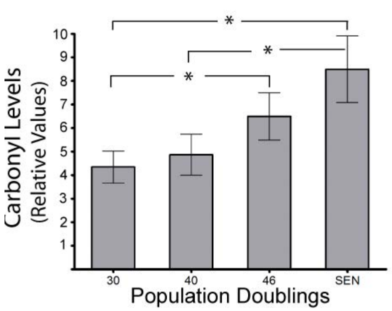
Patent: PCT/EP2012/061749 - WO2012175519

Baraibar et al., J. Proteomics, 2013

Quantification

of oxidized proteins





Advantages:

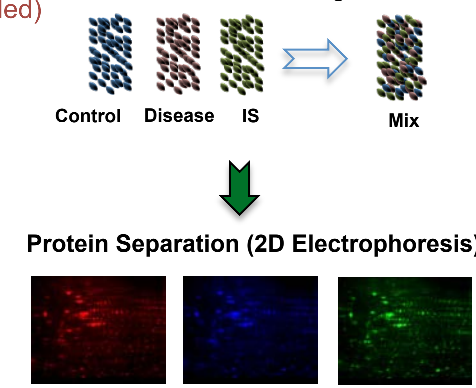
- ✓ **Unprecedented quantification power** (software aided)
- ✓ **High sensitivity & accuracy**
- ✓ **Multiplexing: 3 comparisons per gel**
- ✓ **Less amount of biological material needed**
- ✓ **Reduced inter- and intra-assay variability**
- ✓ **Cost effective**

OXIProteomics

Identification

of specific oxidized proteins

Protein Labelling



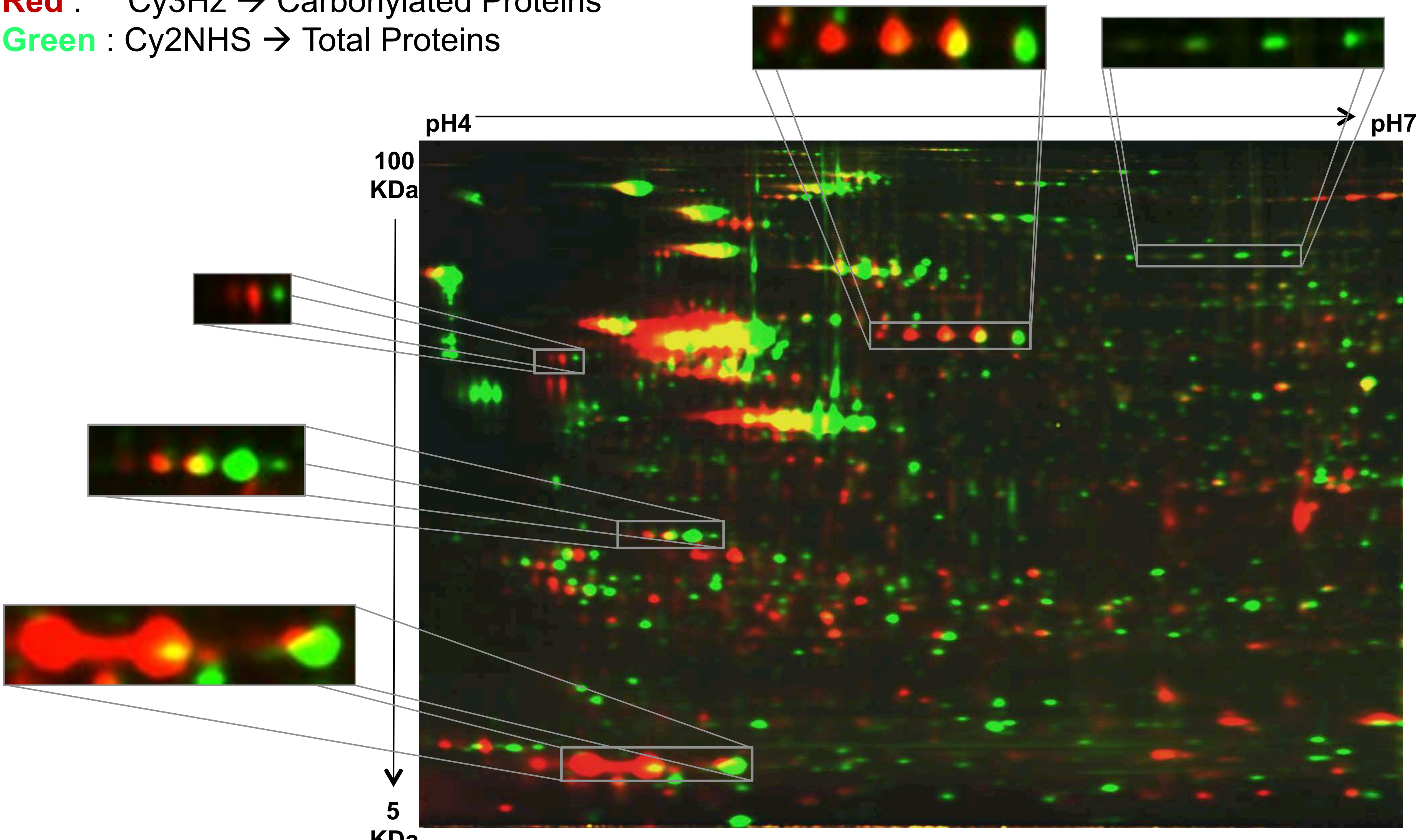
Data Analysis

Disclosure: co-founder and scientific consultant

DETECTION OF CARBONYLATED PROTEINS AFTER 2D SEPARATION

WI-38 fibroblasts upon exposure to H_2O_2 acute oxidative stress

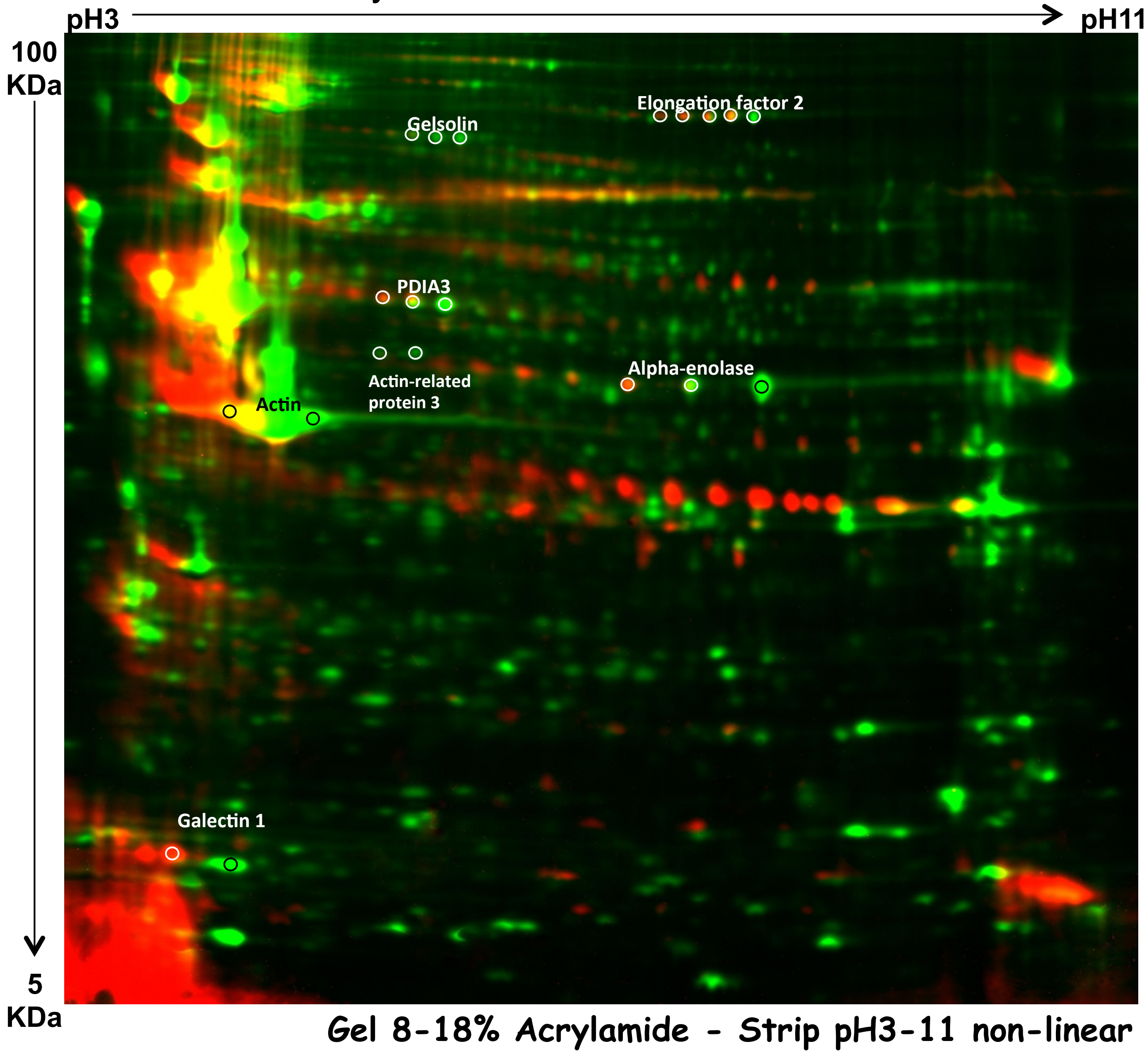
Red : Cy3Hz → Carbonylated Proteins
Green : Cy2NHS → Total Proteins



Gel 8-18% Acrylamide - Strip pH 4-7 Linear

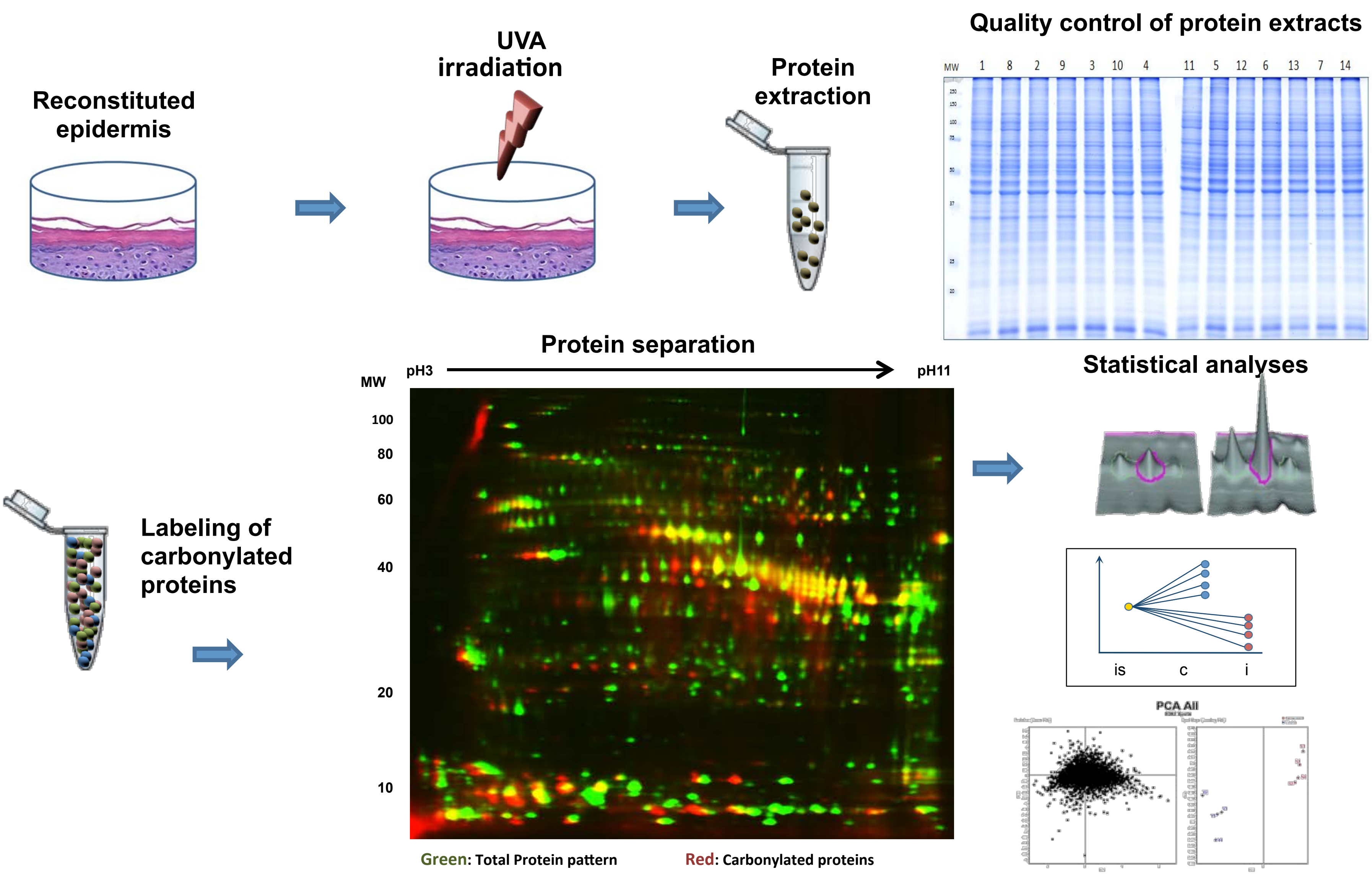
PROTEIN IDENTIFICATION BY MASS-SPECTROMETRY

Red : Cy3Hz → Carbonylated Proteins
Green : Cy2NHS → Total Proteins

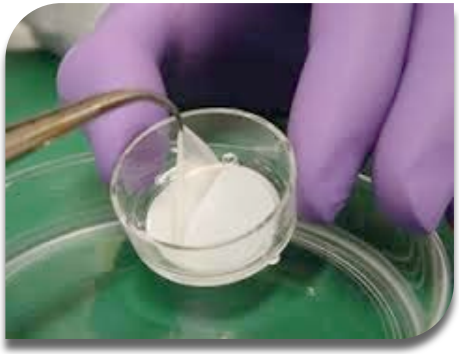


Gel 8-18% Acrylamide - Strip pH3-11 non-linear

VALIDATION FOR PHOTO-AGING APPLICATIONS

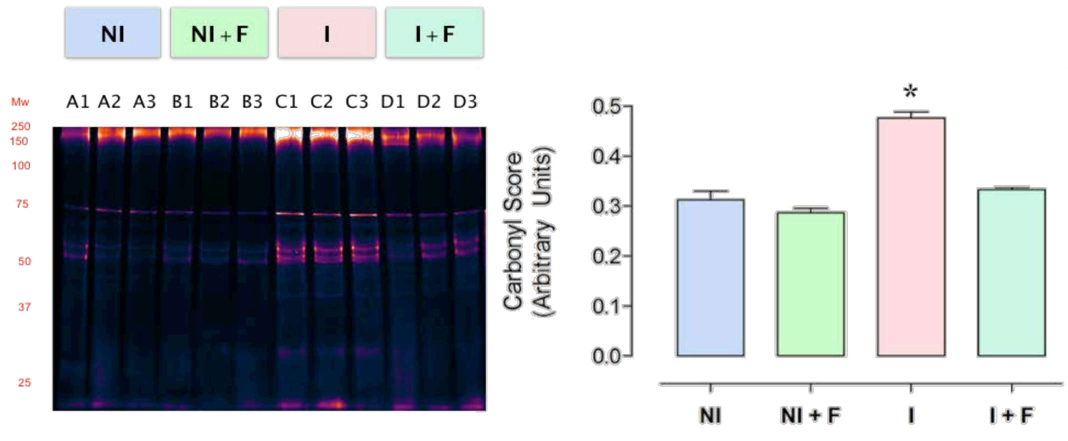


VALIDATION FOR ACTIVE COMPOUNDS EFFICACY TESTING

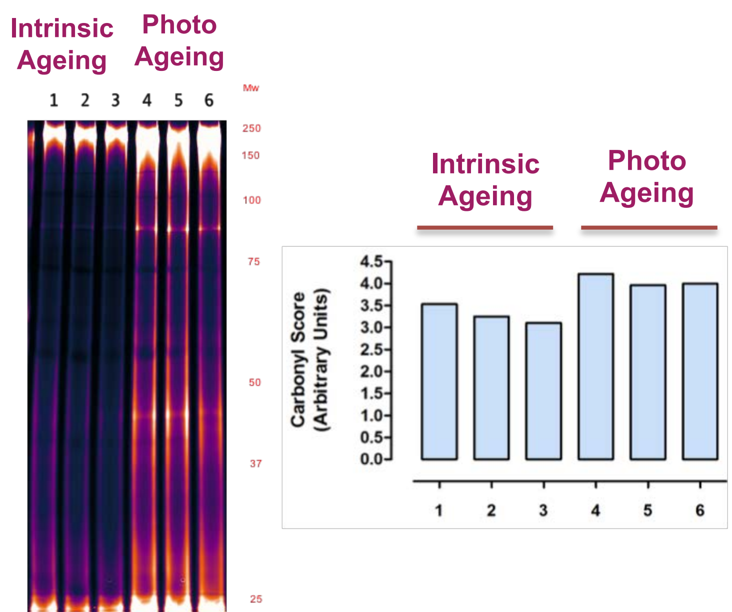


Reconstituted skin

Quantification of oxidized proteins



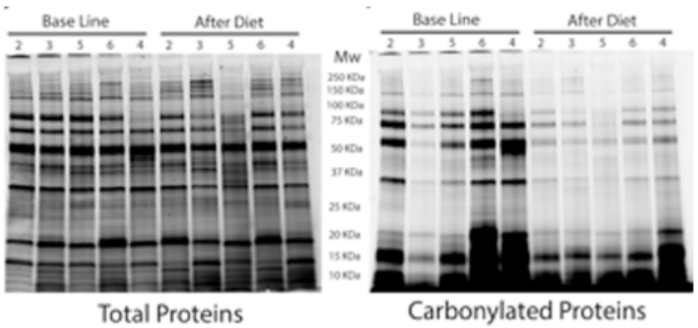
Tape Stripping of *stratum corneum*



OXIProteomics

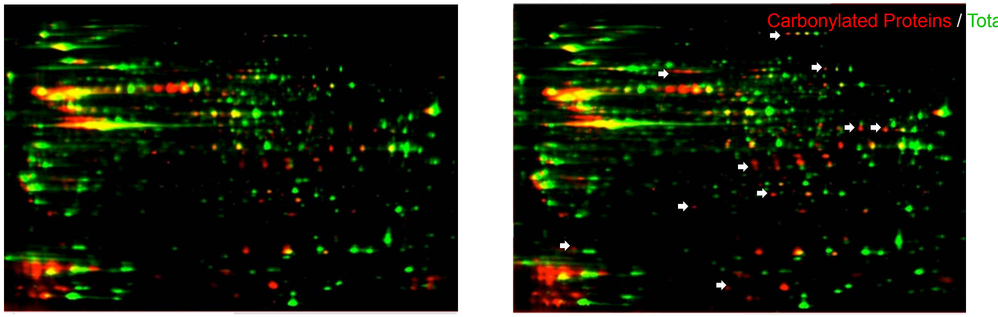
VALIDATION IN BIOLOGICAL SAMPLES FROM DIFFERENT ORIGINS

Seminal Plasma and infertility



Faure et al. 2014, PLoS One

Mitochondrial oxidative stress in cancer cells



Rodier et al. 2015, Cell Reports

INSTITUT DE BIOLOGIE PARIS SEINE

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Germany

Proteomage
EU/FP6 - IP 518230 (2006-11)

MARK AGE
EU/FP7 - IP 200880 (2008-14)

myoage
EU/FP7 - IP 223576 (2009-14)

AFMTELETHON CURE THROUGH INNOVATION
AFM (2013-16)

cost European Cooperation in the field of Scientific and Technical Research
EU/FP7 – COST CM 1001 (2010-14)