

Annamaria Cucinaⁱ, Fabiana Di Gianvincenzoⁱⁱ, David Peggieⁱⁱⁱ, Meaghan Mackie^{ii,iv}, Lucia Burgio^v, Enrico Cappelliniⁱⁱⁱ

ⁱ Laboratory of Organic Mass Spectrometry, Department of Chemical Sciences, University of Catania, Viale A. Doria 6, 95125, Catania, Italy

ⁱⁱ Evolutionary Genomics Section, Globe Institute, University of Copenhagen Oester Voldgade 5-7 1350 Copenhagen K, Denmark

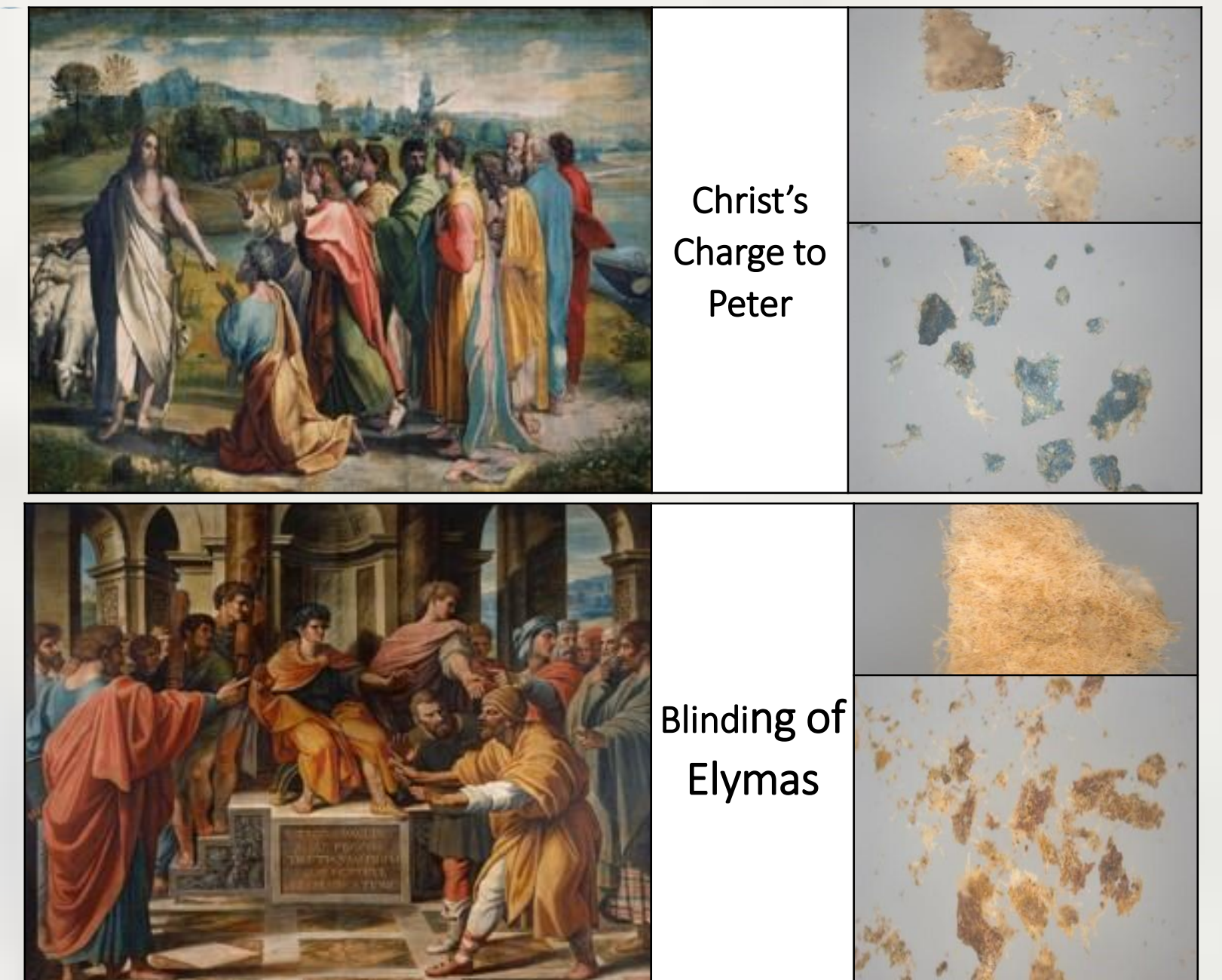
ⁱⁱⁱ National Gallery (Scientific Department), Trafalgar Square, London. WC2N 5DN. UK

^{iv} CPR: Novo Nordisk Foundation Center for Protein Research, Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Blegdamsvej 3B, 6.2, 2200 Copenhagen, Denmark

^v Victoria and Albert Museum, Cromwell Road, London, SW7 2RL. UK

INTRODUCTION

On display in the newly refurbished Raphael Court at the Victoria and Albert Museum are six cartoons by Raphael (on loan from Her Majesty The Queen). Previous conservation work (and associated technical studies) on the cartoons in the 1960s and 1990s had answered many questions regarding the materials used in their construction, but one outstanding issue was the precise composition of the paint medium, which solubility tests and amino acid analysis had indicated might be animal glue. Samples taken previously from three of the cartoons; 'The Death of Ananias' and 'Christ's Charge to Peter' (obtained during the 1960s sampling campaign) and 'The Blinding of Elymas' (obtained during the 1990s sampling campaign) were therefore analysed *via* high resolution mass spectrometry in an attempt to provide more precise information on the proteinaceous content of the paint binder.



METHOD



EXTRACTION
REDUCTION
ALKYLATION



QUANTIFICATION
DIGESTION

PURIFICATION



UHPLC/HR-nESI-MS/MS

Data analysis
Max Quant
software



RESULTS

The results showed the presence of collagens in all samples, with peptides specific to cattle present in every case. The gelatine capsules (used to store the samples from The Death of Ananias and Christ's Charge to Peter) also contained cattle collagens. However, the capsules are unlikely to be a source of contamination since they contain predominantly pig collagens (not observed in the samples). Sheep/goat collagens were observed in the samples from The Blinding of Elymas (and also in the capsules, but at a relatively low number). The high number of sheep/goat peptides in the paper sample compared to the paint sample suggests that they are probably related to the paper rather than the paint binder. The paper samples from all three cartoons also contained a small number of peptides related to fish and/or bird species, while the paint sample in Christ's Charge to Peter also contained egg proteins.

Table 1 Protein and peptides identification for each layer of the cartoons and the gelatine capsules.

Sample	Protein type	Species	All peptides		
The Death of Ananias	Paper (A)	Collagens	<i>Bos taurus</i> Actinopterygii Aves	88 3 2	
		Paint (B)	Collagens	<i>Bos taurus</i>	26
		Christ's Charge to Peter	Paper (C)	Collagens	<i>Bos taurus</i> Aves
Paint (D)	Collagens			<i>Bos taurus</i> <i>Globicephala melas</i>	30 2
	Egg proteins		<i>Gallus gallus</i>	28	
The Blinding of Elymas	Paper (E)	Collagens	<i>Bos taurus</i> <i>Ovis aries</i> <i>Anguilla japonica</i>	8 117 3	
		Paint (F)	Collagens	<i>Bos taurus</i> <i>Ovis aries/Capra hircus</i>	40 77
			Gelatine storage capsules (G)	Collagens	<i>Bos taurus</i> <i>Sus scrofa</i> <i>Equus</i> <i>Ovis aries/Capra hircus</i>

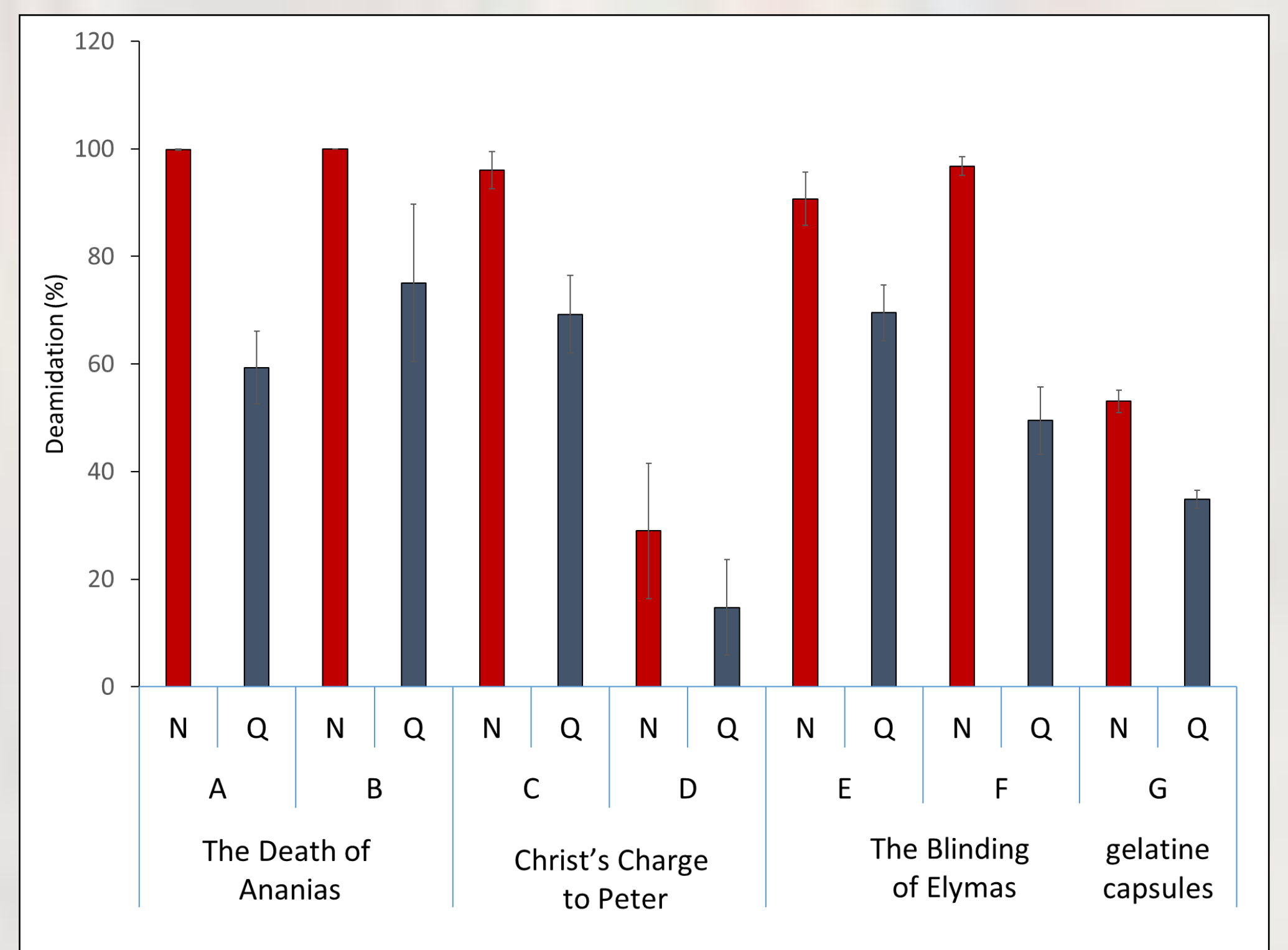


Figure 1: Percentage of deamidation of asparagine (N) and glutamine (Q) amino acids in the six analysed samples and in the gelatine capsules. Error bars indicate confidence interval around 1000 bootstrap replicates.

CONCLUSION

The proteomics results have raised many interesting questions regarding the materials in the samples; was a cattle-based hide glue used as a paint binder in all three samples, or does this reflect the presence of something else? And what is the significance of the small number of bird and fish peptides observed in some samples? In Christ's Charge to Peter, are two separate paints being observed, one bound in hide glue and the other egg tempera? Are they from two separate campaigns and are they both original? Finally, does the presence of sheep and goat peptides in The Blinding of Elymas samples indicate a different intervention, and if so, when? The interpretation of these results is still very much a work in progress.