

Vibrational study on structure and bioactivity of protein fibers grafted with phosphorylated methacrylates

Michele Di Foggia, Masuhiro Tsukada and Paola Taddei

Table S1. Main Raman band wavenumbers and assignments for *B. mori* silk fibroin fabrics before (B m control) and after grafting with phosmer CL, weight gain 26.5% (B m CL 26.5%) and phosmer M, weight gain 22.8% (B m M 22.8%). The bands having a contribution from phosmers are indicated with asterisks (assignments in bold characters).

B m control $\tilde{\nu} / \text{cm}^{-1}$	B m CL 26.5% $\tilde{\nu} / \text{cm}^{-1}$	B m M 22.8% $\tilde{\nu} / \text{cm}^{-1}$	Assignments
	1725 w	1725 w	ester C=O stretching of phosmer [28] *
1667 s	1667 s	1667 s	Amide I of β -sheet conformation [29,30,37]
1616 w	1615 w	1615 w	Trp, Tyr, and Phe of silk fibroin [29,30,37]
1600 w	1599 w	1601 w	Trp and Phe of silk fibroin [29,30,37] and phosphorylated Tyr [36] *
1555 vw	1555 vw	1555 vw	Trp of silk fibroin [29,30,37]
	1485 sh	1485 sh	CH₂ and CH₃ bending of polymerized phosmer [33] *
1451 m	1449 s	1451 s	CH ₂ bending in silk fibroin [29,30,37] and CH₂ and CH₃ bending of grafted phosmer [28] *
1403 w	1403 w	1403 w	C(CH ₃) ₂ antisymmetric bending of silk fibroin [29,30,37] and Ser [41]
1368 vw	1368 vw	1368 vw	Trp of silk fibroin [29,37]
1264 m	1265 m	1266 m	Amide III of unordered conformation [29,30,37]
1229 s	1227 s	1229 s	Amide III of β -sheet conformation [29,30,37]
1171 mw	1171 mw	1171 mw	CC stretching mode of unordered conformation [29,37]
1163 mw	1163 mw	1163 mw	CC stretching mode of β -sheet conformation [29,37]
	1125 vw	1125 vw	CC stretching of polymerized phosmer [31,32] *
1085 s	1085 s	1085 s	CC skeletal stretching mode of β -sheet conformation [29,30,37]
1032 w	1032 w	1034 w	Phe and CC skeletal stretching of silk fibroin [29,37] and CC stretching of polymerized phosmer [31,32] *
1004 w	1004 w	1004 w	Phe of silk fibroin [29,30,37]
979 w	977 w	979 w	CH ₃ rocking mode of β -sheet conformation [29,37]
882 w	882 w	882 w	CH ₂ rocking mode of β -sheet conformation [29,37]
854 w	854 w	854 w	Tyr of silk fibroin [29,30,37] and CH₂ in-plane bending of phosmer [32] *
829 w	829 w	829 w	Tyr of silk fibroin [29,30,37] and CH₂ in-plane bending of phosmer [32] *
	810 sh	810 sh	phosphorylated Tyr and Ser [36] *
760 vw	760 vw	760 vw	Trp of silk fibroin [29,30] and C-Cl and C-C stretching of phosmers [28] *
	706 vw		C-Cl stretching of phosmer CL [28] *
644 w	644 w	644 w	Tyr of silk fibroin [29,37]
622 vw	622 vw	622 vw	Phe of silk fibroin [29]
	603 vw	603 w	CCO symmetric stretching of phosmer [31,32] *

v = very; w = weak; m = medium; s = strong; sh = shoulder.
Trp = tryptophan; Phe = phenylalanine; Tyr = tyrosine, Ser = serine.

Table S2. Main IR band wavenumbers and assignments for *B. mori* silk fibroin fabrics before (B m control) and after grafting with phosmer CL, weight gain 26.5% (B m CL 26.5%) and phosmer M, weight gain 22.8% (B m M 22.8%). The bands having a contribution from phosmers are indicated with asterisks (assignments in bold characters).

B m control $\tilde{\nu} / \text{cm}^{-1}$	B m CL 26.5% $\tilde{\nu} / \text{cm}^{-1}$	B m M 22.8% $\tilde{\nu} / \text{cm}^{-1}$	Assignments
	1732 w	1726 w	ester C=O stretching of phosmer [31,32] *
1697 sh	1697 sh	1697 sh	Amide I of anti-parallel β -sheet conformation [44,45]
	1693 sh	1692 sh	ester C=O stretching of phosmer [31,32] *
1617 s	1617 s	1621 s	Amide I of β -sheet conformation [44,45]
1511 s	1513 s	1511 s	Amide II of β -sheet conformation [44,45]
	1453 sh	1454 sh	CH₂ and CH₃ bending of phosmer [31,32] *
1440 m	1440 m	1440 m	CH ₂ bending in silk fibroin [44,45]
1405 w	1403 vw	1405 vw	Ser of silk fibroin [46,47]
1261 sh			Amide III of unordered conformation [44,45]
	1255 sh	1255 sh	P=O stretching of phosphate group [17,43] *
1226 m			Amide III of β -sheet conformation [44,45]
1163 m	1161 m	1161 m	CC stretching in silk fibroin [44,45]
	1152 sh	1146 sh	COC antisymmetric stretching of phosmer [31,32] and P-O-C stretching [17,43] *
1030 sh	1036 m	1036 m	CC stretching in silk fibroin [44,45] and P-O stretching [17,43] *
	999 vw	1001 vw	P-O stretching [17,43] *
997 vw	997 sh	997 sh	Ala-Gly sequences in silk fibroin [44,45]
976 vw	976 vw	976 vw	Ala-Gly sequences in silk fibroin [44,45]
	880 vw	877 vw	CH₂ rocking of phosmer [31] *
	830 vw	830 vw	P-O-C bending [31] *

v = very; w = weak; m = medium; s = strong; sh = shoulder.
Ser = serine.

Table S3. Main Raman band wavenumbers and assignments for Tussah silk fibroin fabrics before (T control) and after grafting with phosmer CL, weight gain 13.7% (T CL 13.7%) and phosmer M, weight gain 12.4% (T M 12.4%). The bands having a contribution from phosmers are indicated with asterisks (assignments in bold characters).

T m control $\tilde{\nu} / \text{cm}^{-1}$	T CL 13.7% $\tilde{\nu} / \text{cm}^{-1}$	T M 12.4% $\tilde{\nu} / \text{cm}^{-1}$	Assignments
	1720 w	1720 w	ester C=O stretching of phosmer [28] *
1668 s	1668 s	1668 s	Amide I of β -sheet conformation [48-50]
1614 w	1614 w	1614 w	Trp, Tyr, and Phe of silk fibroin [29]
1600 w	1599 w	1599 w	Trp and Phe of silk fibroin [29] and phosphorylated Tyr [36] *
1555 vw	1555 vw	1552 vw	Trp of silk fibroin [29]
1452 m	1450 s	1450 s	CH₂ bending in silk fibroin [29] and CH₂ and CH₃ bending of grafted phosmer [28] *
1398 w	1398 w	1398 w	C(CH ₃) ₂ antisymmetric bending of silk fibroin [29] and Ser [41]
1365 vw	1365 vw	1365 vw	Trp of silk fibroin [29]
1270÷1260 sh br	1270÷1260 sh br	1270÷1260 sh br	Amide III of α -helix and unordered conformations [48-50]
1242-1230 s	1242-1230 s	1242-1230 s	Amide III of β -sheet conformation [48-50]
	1128 w	1128 w	CC stretching of polymerized phosmer [31,32] *
1094 s	1094 s	1094 s	Ala-rich sequences in β -sheet conformation [48-50]
1069 s	1069 s	1069 s	Ala-rich sequences in β -sheet conformation [48-50]
1032 w	1036 w	1036 w	Phe and CC skeletal stretching of silk fibroin [29] and CC stretching of polymerized phosmer [31,32] *
1013 w	1009 w	1009 w	Phe of silk fibroin [29] and CC stretching of polymerized phosmer [31,32] *
965 m	965 m	965 m	Ala-rich sequences in β -sheet conformation [48-50]
907 s	907 s	907 s	Ala-rich sequences in β -sheet conformation [48-50]
854 w	854 w	854 w	Tyr of silk fibroin [29,30,37]
829 w	829 w	829 w	Tyr of silk fibroin [29,30,37]
760 vw	761 vw	761 vw	Trp of silk fibroin [29] and C-Cl and C-C stretching of phosmers [28] *
644 w	644 w	644 w	Tyr of silk fibroin [29]

v = very; w = weak; m = medium; s = strong; sh = shoulder; br = broad.
Trp = tryptophan; Phe = phenylalanine; Tyr = tyrosine, Ser = serine.

Table S4. Main IR band wavenumbers and assignments for Tussah silk fibroin fabrics before (T control) and after grafting with phosmer CL, weight gain 13.7% (T CL 13.7%) and phosmer M, weight gain 12.4% (T M 12.4%). The bands having a contribution from phosmers are indicated with asterisks (assignments in bold characters).

T control $\tilde{\nu}$ / cm^{-1}	T CL 13.7% $\tilde{\nu}$ / cm^{-1}	T M 12.4% $\tilde{\nu}$ / cm^{-1}	Assignments
	1732-1717 w	1732-1717 w	ester C=O stretching of phosmer [31,32] *
1695 sh	1695 sh	1695 sh	Amide I of anti-parallel β -sheet conformation [48]
1622 s	1622 s	1624 s	Amide I of β -sheet conformation [52]
1511 s	1513 s	1513 s	Amide II of β -sheet conformation [52]
	1453 sh	1454 sh	CH₂ and CH₃ bending of phosmer [31,32] *
1444 m	1444 m	1444 m	CH ₂ bending in silk fibroin [52] and CH₂ and CH₃ bending of phosmer [31,32] *
1242-1222 m	1242-1218 m	1242-1218 m	Amide III of β -sheet conformation [48,49]
1165 m	1165-1134 m	1165-1134 m	CC stretching in silk fibroin [44,45] and COC antisymmetric stretching of phosmer [31,32] and P-O-C stretching [17,43] *
1030 sh	1034 mw	1034 mw	CC stretching in silk fibroin [44,45] and P-O stretching [17,43] *
	1006 mw	1006 mw	P-O stretching [17,43] *
963 mw	963 mw	963 mw	CH ₃ rocking in a β -sheet conformation [49] or Ala-Ala linkages [53]
	830 w	830 w	P-O-C bending [31] *
673 vw			unordered conformations [53]

v = very; w = weak; m = medium; s = strong; sh = shoulder.
Ser = serine.

Table S5. Main Raman band wavenumbers and assignments for wool keratin fabrics before (W control) and after grafting with phosmer CL, weight gain 26.6% (W CL 26.6%) and phosmer M, weight gain 12.7% (W M 12.7%). The bands having a contribution from phosmers are indicated with asterisks (assignments in bold characters), those assignable to oxidation products are indicated with triangles (assignments in italic characters).

W m control $\tilde{\nu} / \text{cm}^{-1}$	W CL 26.6% $\tilde{\nu} / \text{cm}^{-1}$	W M 12.7% $\tilde{\nu} / \text{cm}^{-1}$	Assignments
	1725 w	1725 w	ester C=O stretching of phosmer [28] *
	1705 sh	1705 sh	ester C=O stretching of phosmer [28] *
1673 sh	1673 sh	1673 sh	Amide I of β -sheet/unordered conformation [55-57]
1655 s	1655 s	1655 s	Amide I of α -helix conformation [55-57]
1614 w	1614 w	1614 w	Trp, Tyr, and Phe of wool keratin [55-57]
1607 sh	1601 s	1601 s	Trp and Phe of wool keratin [55-57] and phosphorylated Tyr [33] *
1588 w	1588 sh	1588 sh	Phe of wool keratin [55-57]
1553 vw	1553 vw	1553 vw	Trp of wool keratin [55-57]
	1485 sh	1485 sh	CH₂ and CH₃ bending of polymerized phosmer [33] *
1451 m	1451 s	1451 s	CH ₂ bending in wool keratin [55-57] and CH₂ and CH₃ bending of grafted phosmer [28] *
1398 w	1398 vw	1398 vw	C(CH ₃) ₂ antisymmetric bending in wool keratin [55-57] and Ser [41]
1272 m	1272 m	1272 m	Amide III of α -helix conformation [55-57]
1249 m	1249 m	1249 m	Amide III of β -sheet/unordered conformation [55-57]
1127 m	1127 m	1127 m	CC stretching of wool keratin [55-57] and CC stretching of polymerized phosmer [31,32] *
1098 w	1094 m	1094 m	CC stretching of wool keratin [55-57] and <i>SO₂ symmetric stretching [47,62] ▲</i>
1032 w	1037 s	1037 s	Phe and CC stretching of wool keratin [55-57] and <i>cysteic acid [59-61] ▲</i>
1004 s	1004 s	1004 s	Phe of wool keratin [55-57]
936 w	934 w	934 w	α -helix conformation [58]
854 w	854 w	854 w	Tyr of wool keratin [55-57]
829 w	829 w	829 w	Tyr of wool keratin [55-57]
760 vw	760 m	760 m	Trp of wool keratin [55-57] and C-Cl and C-C stretching of phosmers [28] *
	705 vw		C-Cl stretching of phosmer CL [28] *
644 w	644 w	644 w	Tyr of wool keratin [55-57]
622 w	622 w	622 w	Phe of wool keratin [55-57]
	570 sh	570 sh	<i>SO₃ bending [47,62] ▲</i>
547 sh	547 w	547 w	SS bridges, trans-gauche-trans conformation [42,63,64]
520 sh	520 sh	520 sh	SS bridges, gauche-gauche-trans conformation [42,63,64]
510 mw	510 mw	510 mw	SS bridges, gauche-gauche-gauche conformation [42,63,64]
494 sh	494 sh	494 sh	SS bridges, strained conformation [42,63,64]
430 sh	430 w	430 w	<i>SO₃ rocking [47,62] ▲</i>
412 vw	412 w	412 w	<i>SO₃ rocking [47,62] ▲</i>

v = very; w = weak; m = medium; s = strong; sh = shoulder.
Trp = tryptophan; Phe = phenylalanine; Tyr = tyrosine, Ser = serine.

Table S6. Main IR band wavenumbers and assignments for wool keratin fabrics before (W control) and after grafting with phosmer CL, weight gain 26.6% (W CL 26.6%) and phosmer M, weight gain 12.7% (W M 12.7%). The bands having a contribution from phosmers are indicated with asterisks (assignments in bold characters), those assignable to oxidation products are indicated triangles (assignments in italic characters).

W control $\tilde{\nu} / \text{cm}^{-1}$	W CL 26.6% $\tilde{\nu} / \text{cm}^{-1}$	W M 12.7% $\tilde{\nu} / \text{cm}^{-1}$	Assignments
	1734-1716-1696 w	1734-1716-1696 w	ester C=O stretching of phosmer [31,32] *
1646 sh	1646 sh	1646 sh	Amide I of α -helix conformation [65]
1626 s	1626 s	1626 s	Amide I of β -sheet conformation [55,64]
1537 sh	1537 sh	1537 sh	Amide II of α -helix conformation [65]
1512 s	1515-1519 s	1513 s	Amide II of β -sheet conformation [55,64]
1444 m	1454-1441 sh	1451 sh	CH ₂ bending in wool keratin [55,64] and CH₂ and CH₃ bending of phosmer [31,32] *
1387 m			OH bending of Ser [47]
1302 sh			Amide III of α -helix conformation [65]
1230 m			Amide III of β -sheet conformation [55,64]
	1203 m	1203 m	<i>SO₂ antisymmetric stretching of alkyl and aryl sulphates [47], sulfoamines and sulfoamides [62] ▲</i>
1169 w	1166 ms	1166 ms	<i>cysteic acid [66,67] ▲</i>
	1127 w	1127 w	<i>cystine dioxide [68] ▲</i>
	1074 sh	1074 sh	<i>cystine monoxide [68] ▲</i>
1069 m, br			CO stretching of Ser [47]
	1034 s	1034 s	<i>cysteic acid [66,67] ▲</i>
	1003 ms	1005 ms	<i>CO stretching of organic sulphates [47] ▲</i>
	832 vw	832 vw	P-O-C bending [31] *
	748 vw	748 vw	<i>SOC symmetric stretching of organic sulphates [47] ▲</i>
	652 vw	652 vw	<i>SO₃ bending of organic sulphates [47] ▲</i>
v = very; w = weak; m = medium; s = strong; sh = shoulder. Ser = serine.			

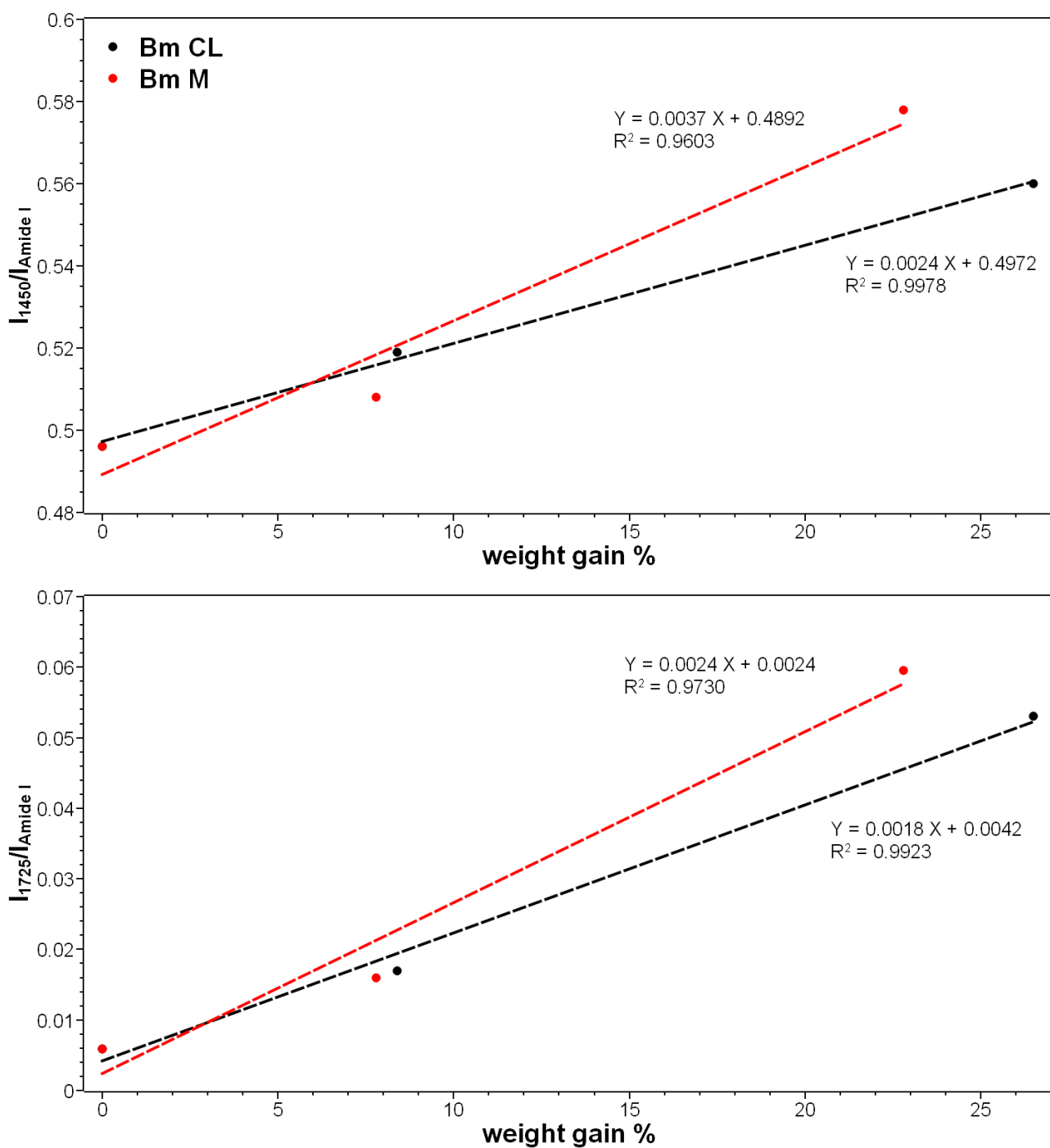


Figure S1. Trend of the $I_{1450}/I_{Amide\ I}$ and $I_{1725}/I_{Amide\ I}$ Raman intensity ratios as a function of the weight gain for the *B. mori* silk fibroin fabrics grafted with phosmer CL (black) and phosmer M (red), respectively.

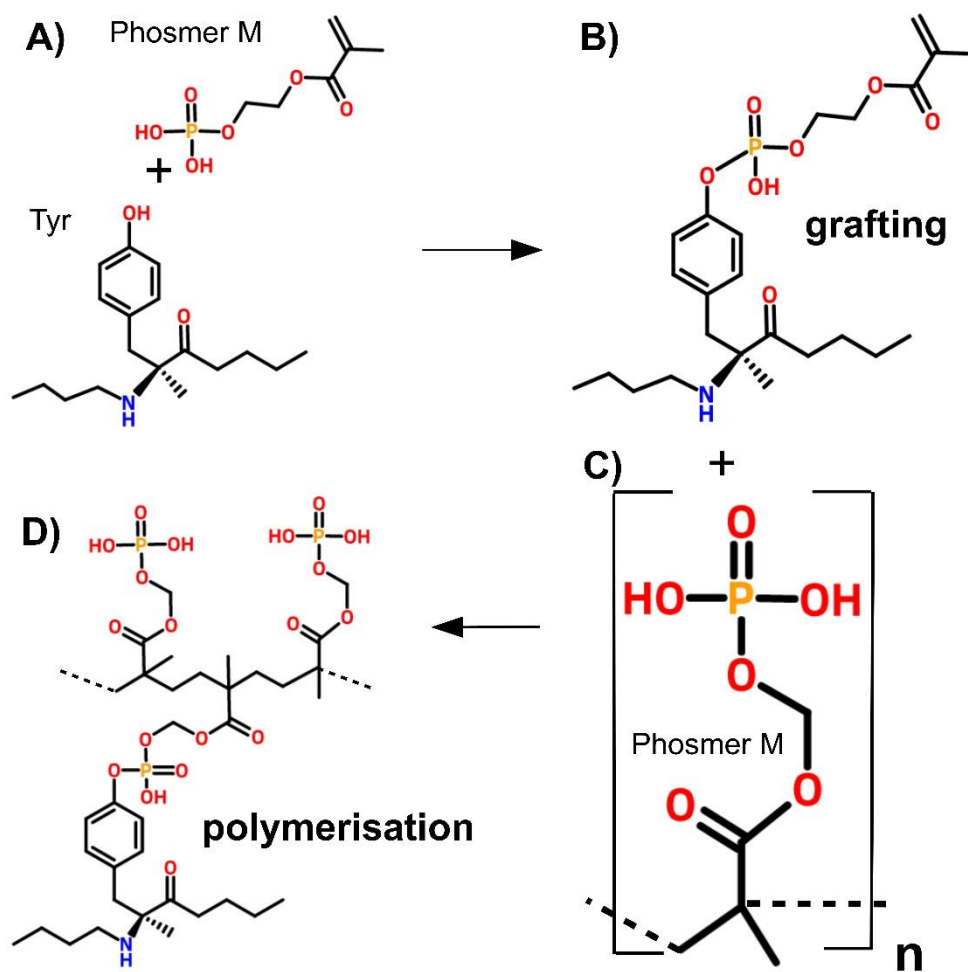


Figure S2. Reaction scheme of the functionalization of silk fibroin with phosmer M: A) Phosmer M reacts with silk fibroin (tyrosine is a preferential site); B) grafting reaction between a phosmer M molecule and the Tyr side chain of silk fibroin; C) and D) polymerization.

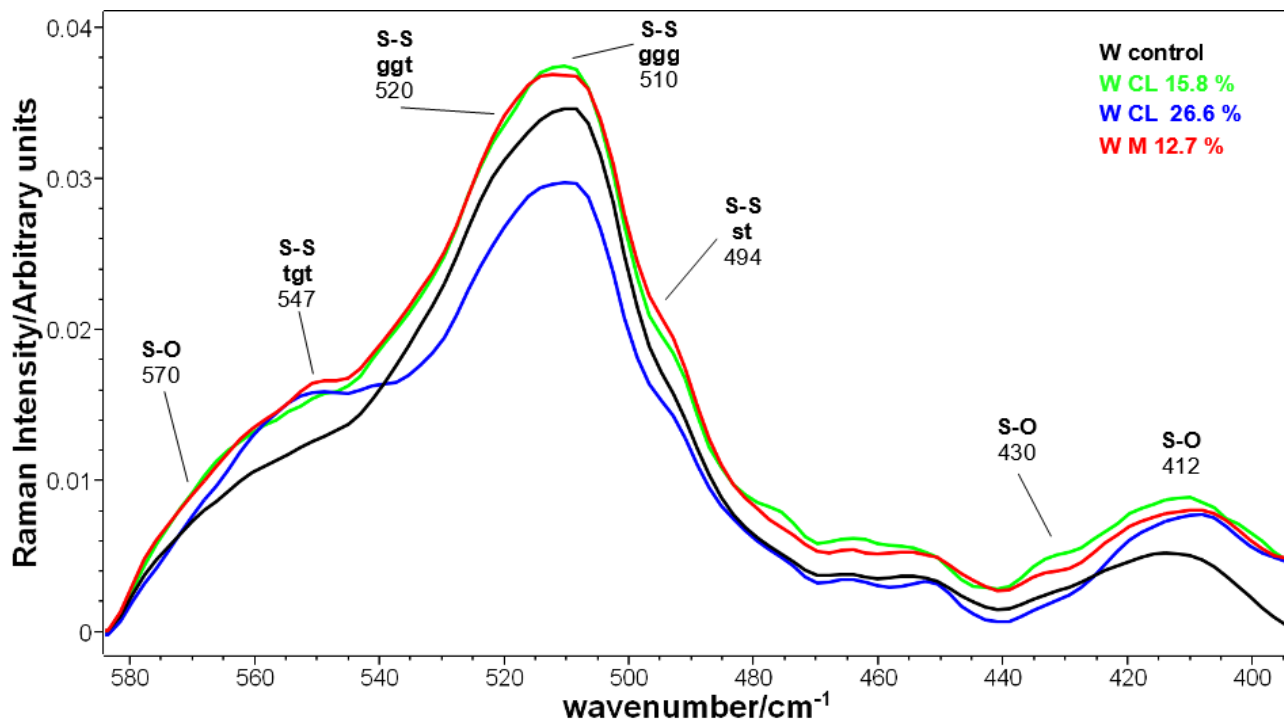


Figure S3. Raman spectra of wool keratin fabrics before (control) and after grafting with phosmer CL (weight gains 15.8% and 26.6%) and M (weight gain 12.4%). The spectra are normalized to the Amide I band. The assignments to the main conformations adopted by disulfide bridges (S-S), i.e. strained (st), gauche-gauche-gauche (ggg), gauche-gauche-trans (ggt) and trans-gauche-trans (tgt), as well to sulfated species (S-O) are indicated.

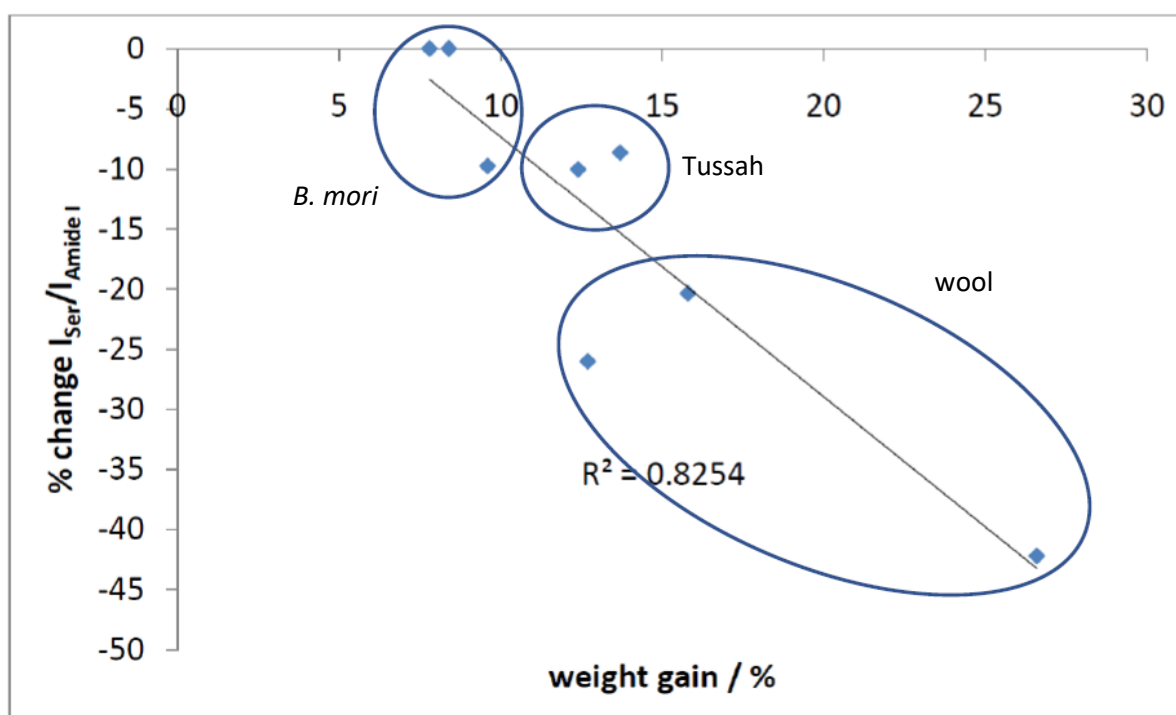
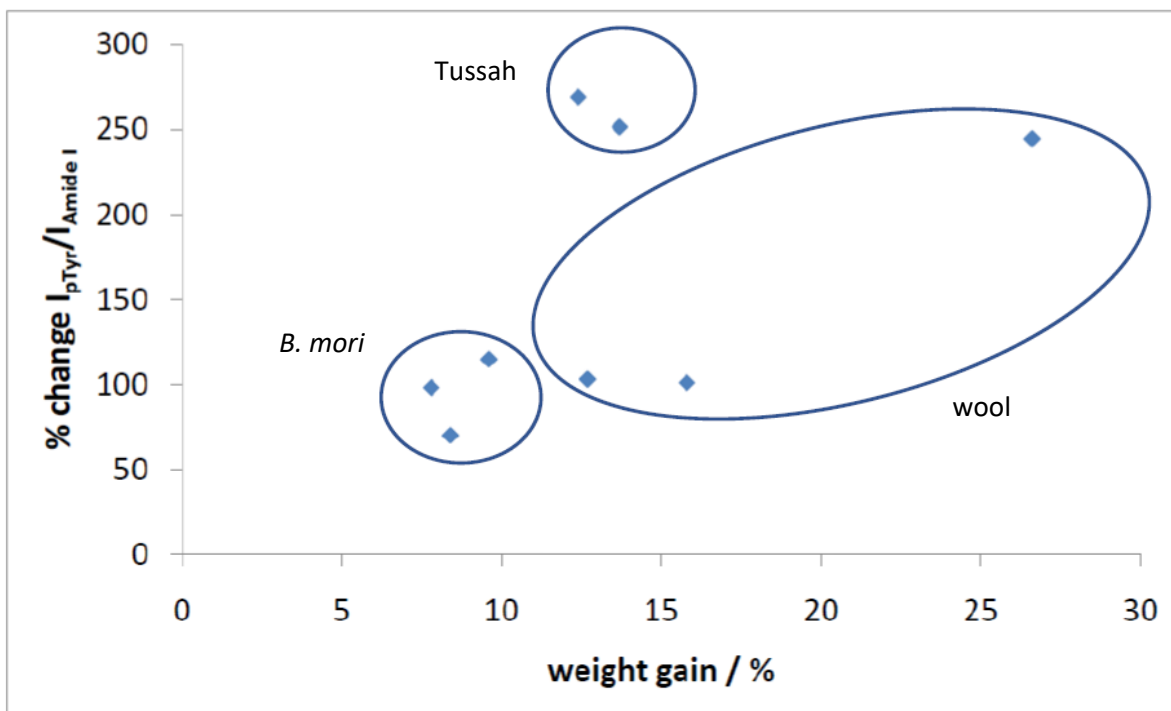


Figure S4. Trend of the % change of the $I_{pTyr}/I_{Amide I}$ ratio (top) and $I_{Ser}/I_{Amide I}$ (bottom) upon grafting as a function of weight gain for *B. mori* (with the exception of the degraded B m CL 26.5% and B m M 22.8%), Tussah silk fibroin and wool fabrics.

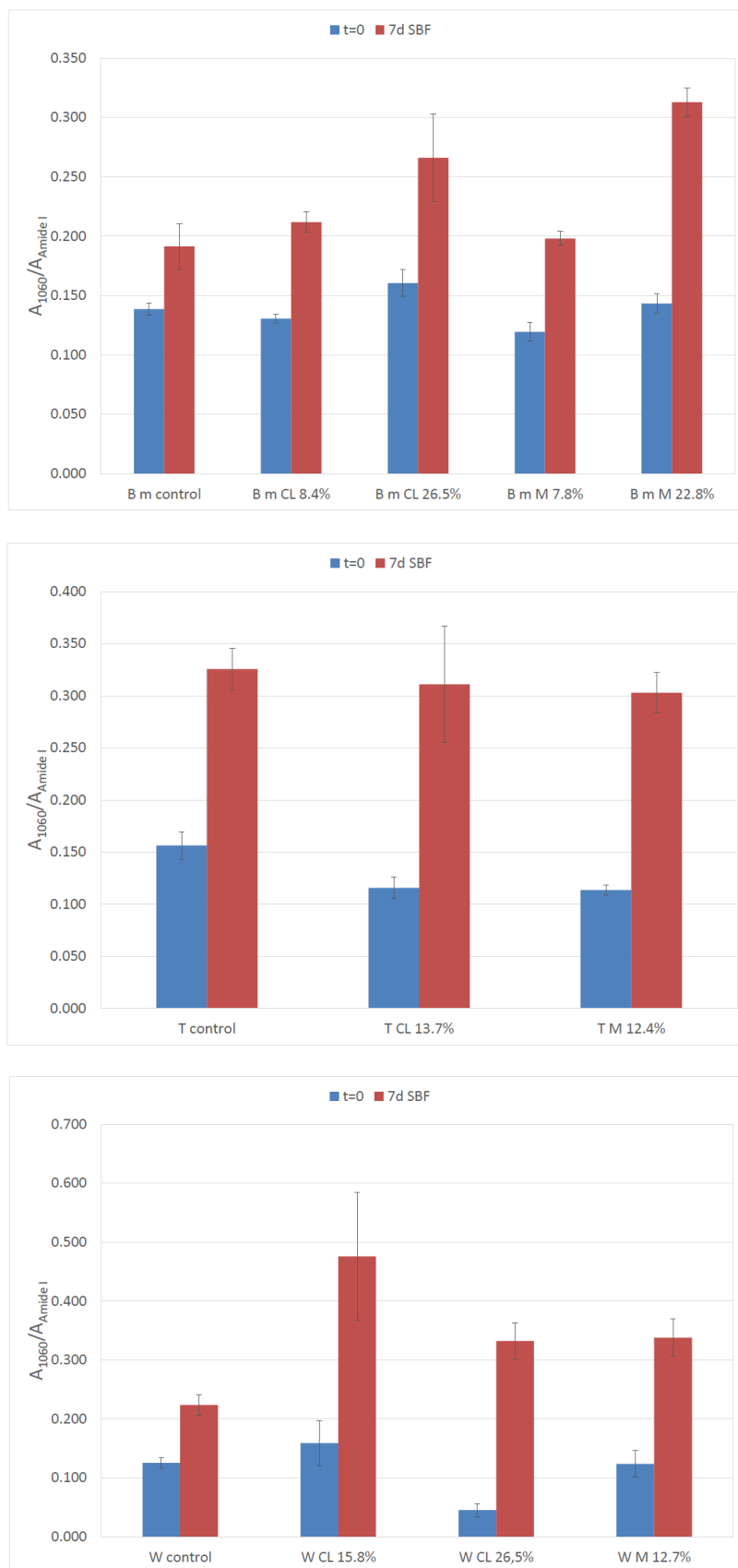


Figure S5. Values of the $A_{1060}/A_{\text{Amide I}}$ absorbance ratio (average \pm standard deviation), as obtained from the IR spectra of the samples before (t=0) and after bioactivity tests (7d SBF).

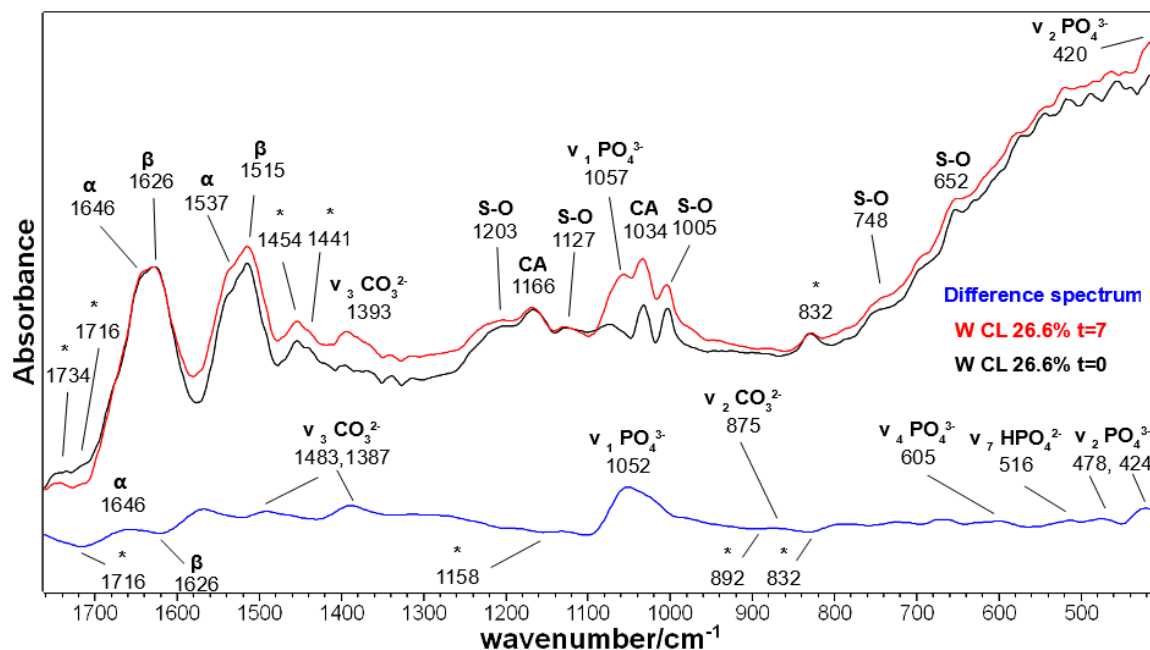


Figure S6. IR spectra of wool CL 26.6% fabric before (black) and after (red) immersion in an SBF solution for 7 days. The spectra are normalized to the Amide I band. Difference spectrum was calculated by subtracting the spectrum at $t=0$ from that at $t=7$ d. Asterisks indicate the main spectral features ascribable to phosmer. The main bands attributed to β -sheet (β) and α -helix (α) conformations, as well as to cysteic acid (CA), and other sulfur oxidation products (S-O), carbonate and phosphate are indicated.

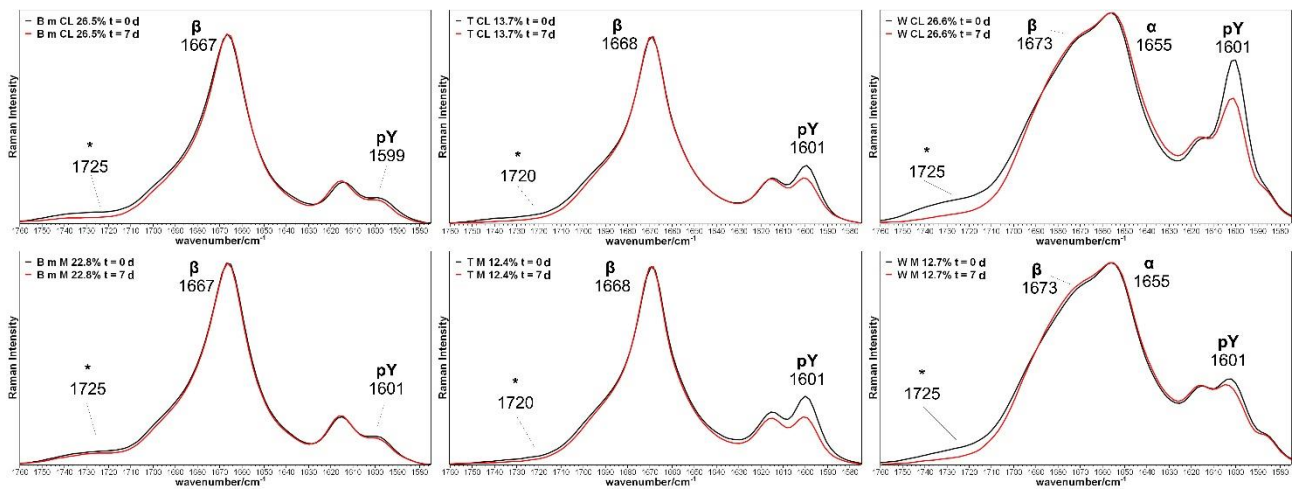


Figure S7. Raman spectra of selected grafted fabrics before (black) and after 7 days immersion in SBF (red). The spectra are normalized to the Amide I band. The main bands assignable to β -sheet (β) or α -helix (α) conformation as well as phosphorylated tyrosine (pY), are indicated.

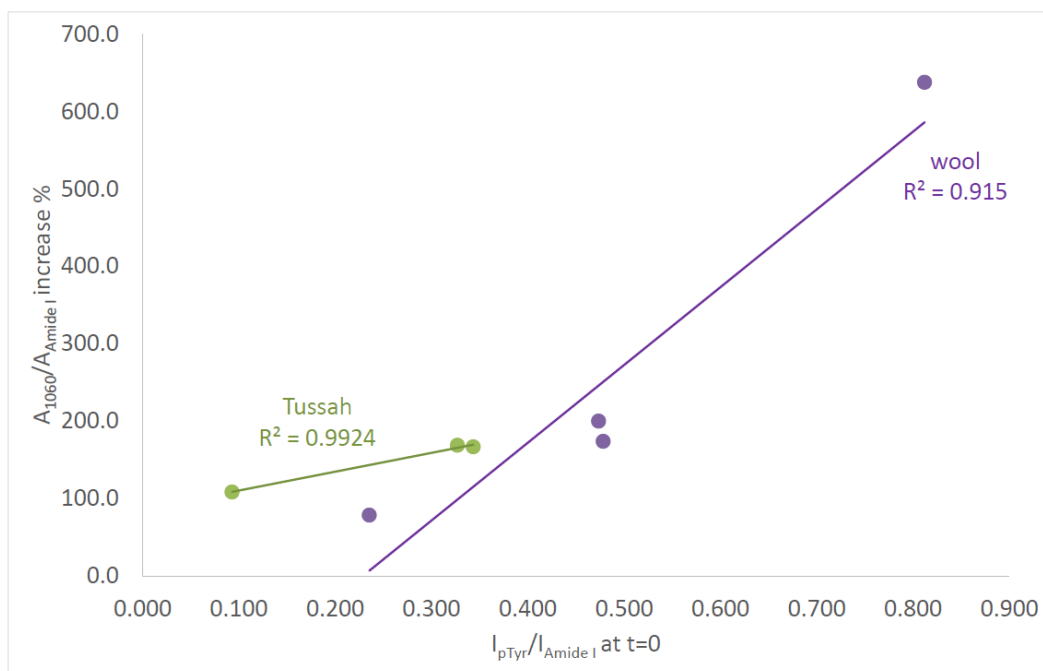


Figure S8. Trend of the $A_{1060}/A_{\text{amide I}}$ IR absorbance ratio increase % after 7 days immersion in the SBF solution and the Raman $I_{\text{pTyr}}/I_{\text{amide I}}$ intensity ratios of the starting materials (i.e., at $t=0$), for Tussah and wool fabrics.