Biotechnology Journal

Supporting Information for DOI 10.1002/biot.201300207

The human anti-HIV antibodies 2F5, 2G12 and PG9 differ in their susceptibility to proteolytic degradation: Down-regulation of endogenous serine and cysteine proteinase activities could improve antibody production in plant-based expression platforms

Melanie Niemer, Ulrich Mehofer, Juan Antonio Torres Acosta, Maria Verdianz, Theresa Henkel, Andreas Loos, Richard Strasser, Daniel Maresch, Thomas Rademacher, Herta Steinkellner and Lukas Mach

Table S1. Sequences of 2F5, 2G12 and PG9 heavy and light chains. CDR H3 loops (green), V_H-C_H1 linker segments (turquoise) and C_H1-C_H2 hinge regions (yellow) are highlighted by coloured backgrounds. Identified cleavage sites are indicated by red arrows.

2F5 (heavy chain):
RITLKESGPPLVKPTQTLTLTCSFSGFSLSDFGVGVGWIRQPPGKALEWLAIIYSDDDKRYSPSLNTRLTIT
KDTSKNQVVLVMTRVSPVDTATYFCAHRRGPTTLF↓G↓VPIARGPVNAMDVWGQGITVTIS↓STSTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSL
GTQTYICNVNHKPSNTKVD↓K↓KVEPKSCD↓KTH↓TCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN
KAFPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

2F5 (light chain):
ALQLTQSPSSLSASVGDRITITCRASQGVTSALAWYRQKPGSPPQLLIYDASSLESGVPSRFSGSGSGTEF
TLTISTLRPEDFATYYCQQLHFYPHTFGGGTRVDVRRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF
NRGEC

2F5-KDEL (heavy chain):
RITLKESGPPLVKPTQTLTLTCSFSGFSLSDFGVGVGWIRQPPGKALEWLAIIYSDDDKRYSPSLNTRLTIT
KDTSKNQVVLVMTRVSPVDTATYFCAHRRGPTTLFGVPIARGPVNAMDVWGQGITVTISSTSTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV
SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAFPAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKSEKDEL

2F5-KDEL (light chain):
ALQLTQSPSSLSASVGDRITITCRASQGVTSALAWYRQKPGSPPQLLIYDASSLESGVPSRFSGSGSGTEF
TLTISTLRPEDFATYYCQQLHFYPHTFGGGTRVDVRRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF
NRGECSEKDEL

2G12 (heavy chain):
EVQLVESGGGLVKAGGSLILSCGVSNFRISAHTMNWVRRVPGGGLEWVASISTSSTYRDYADAVKGRF
TVSRDDLEDFVYLQMHKMRVEDTAIYYCARKGSDRLSDNDPFDAWGPG↓TVVTVSPASTKGPSVFPLA
PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI
CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAFPAPIEKT
ISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

2G12 (light chain):
AVVMTQSPSTLSASVGDTITITCRASQSIETWLAWYQQKPGKAPKLLIYKASTLKTGVPSRFSGSGSGTE
FTLTISGLQFDDFATYHCQHYAGYSATFGQGTRVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY
PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS
FNRGEC

PG9 (heavy chain):
QRLVESGGGVVQPGSSLRLSCAASGFDFSRQGMHWVRQAPGQGLEWVAFIKYDGSEKYHADSVWGR
LSISRDNSKDTLYLQMNSLRVEDTATYFCVREAGGPDYRNGYNYY↓D↓FYDGYYNYHYMDVWGKGT
TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS
LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP
ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

PG9 (light chain):
QSALTQPASVSGSPGQSITISCQGTSNDVGGYESVSWYQQHPGKAPKVVIYDVSKRPSGVSNRFSGSKS
GNTASLTISGLQAEDEGDYYCKSLTSTRRRVFGTGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCL

ISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHKSYSCQVTHEGSTVE
KTVAPTECS

PG9-KDEL (heavy chain):
QRLVESGGGVVQPGSSLRLSCAASGFDFSRQGMHWVRQAPGQGLEWVAFIKYDGSEKYHADSVWGR
LSISRDNSKDTLYLQMNSLRVEDTATYFCVREAGGPDYRNGYNYYDFYDGYYNYHYMDVWGKGTTV
TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS
SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI
SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN
NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDEL

PG9-KDEL (light chain):
QSALTQPASVSGSPGQSITISCQGTSNDVGGYESVSWYQQHPGKAPKVVIYDVSKRPSGVSNRFSGSKS
GNTASLTISGLQAEDEGDYYCKSLTSTRRRVFGTGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCL
ISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHKSYSCQVTHEGSTVE
KTVAPTECSKDEL

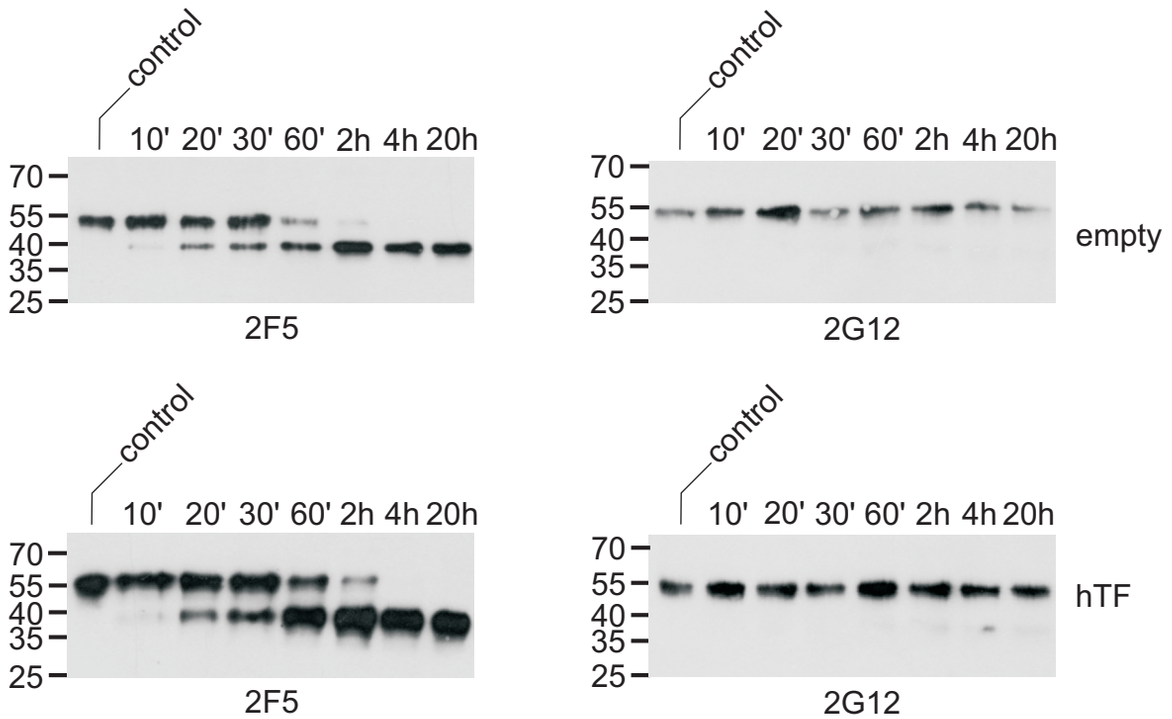

Figure S1. Processing of 2F5 and 2G12 by *N. benthamiana* proteinases *in vitro*. CHO-derived 2F5 or 2G12 (200 ng) was incubated with intercellular fluid (650 ng protein) prepared from leaves infiltrated with *A. tumefaciens* carrying an expression vector for human transferrin (hTF) or the parental bacterial strain (empty) for the indicated times and then analysed by immunoblotting with antibodies to the heavy chain of human IgG. Untreated antibody was loaded as control. The migration positions of selected molecular mass standards are indicated, with their respective masses expressed in kDa.

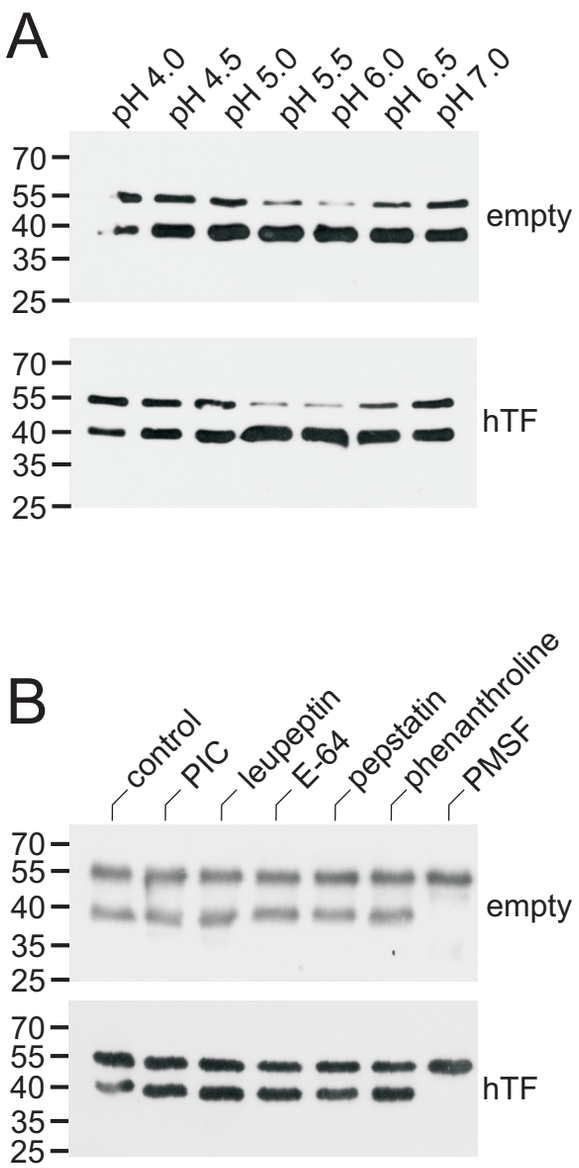

Figure S2. Effect of pH and proteinase inhibitors on *in vitro* processing of 2F5. **(A)** CHO-derived 2F5 (200 ng) was incubated with intercellular fluid (650 ng protein) prepared from leaves infiltrated with *A. tumefaciens* carrying an expression vector for human transferrin (hTF) or the parental bacterial strain (empty) for 2 h at the indicated pH and then analysed by immunoblotting with antibodies to the heavy chain of human IgG. **(B)** CHO-derived 2F5 (200 ng) was incubated with intercellular fluid (IF; 330 ng protein) for 2 h in the absence (control) or presence of the indicated proteinase inhibitors (10 µM: leupeptin, E-64, pepstatin; 2 mM: PMSF, phenanthroline) and then analysed as above. PIC, proteinase inhibitor cocktail. The migration positions of selected molecular mass standards are indicated, with their respective masses expressed in kDa.

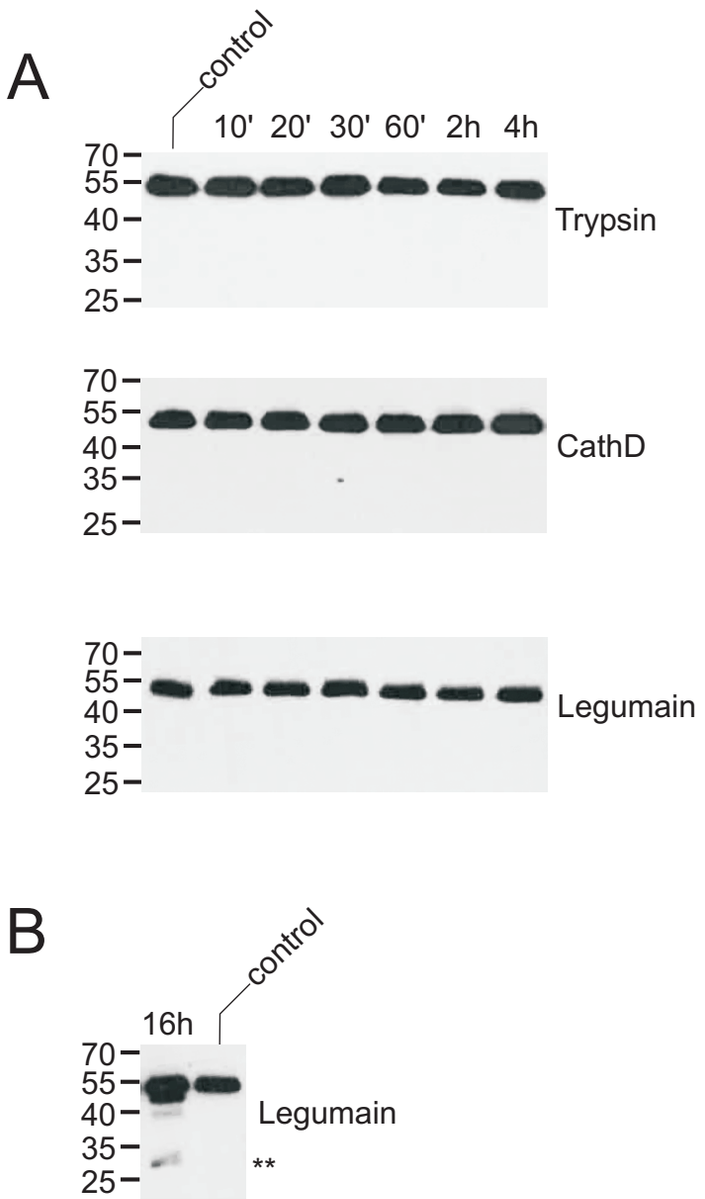

Figure S3. Processing of 2F5 *in vitro*. (**A**) CHO-derived 2F5 (200 ng) was incubated with selected proteinases (10 ng) at pH 5.5 for the indicated times and then analysed by immunoblotting with antibodies to the heavy chain of human IgG. Untreated antibody was used as a control. CathD, cathepsin D. (**B**) CHO-derived 2F5 (200 ng) was incubated with legumain (250 ng) for 16 h at pH 5.5 and then analysed as above. Untreated antibody was used as a control. **, 30-kDa degradation product. The migration positions of selected molecular mass standards are indicated, with their respective masses expressed in kDa.