

Protein Corona of functionalized targeted nanoparticles.

A comparative study

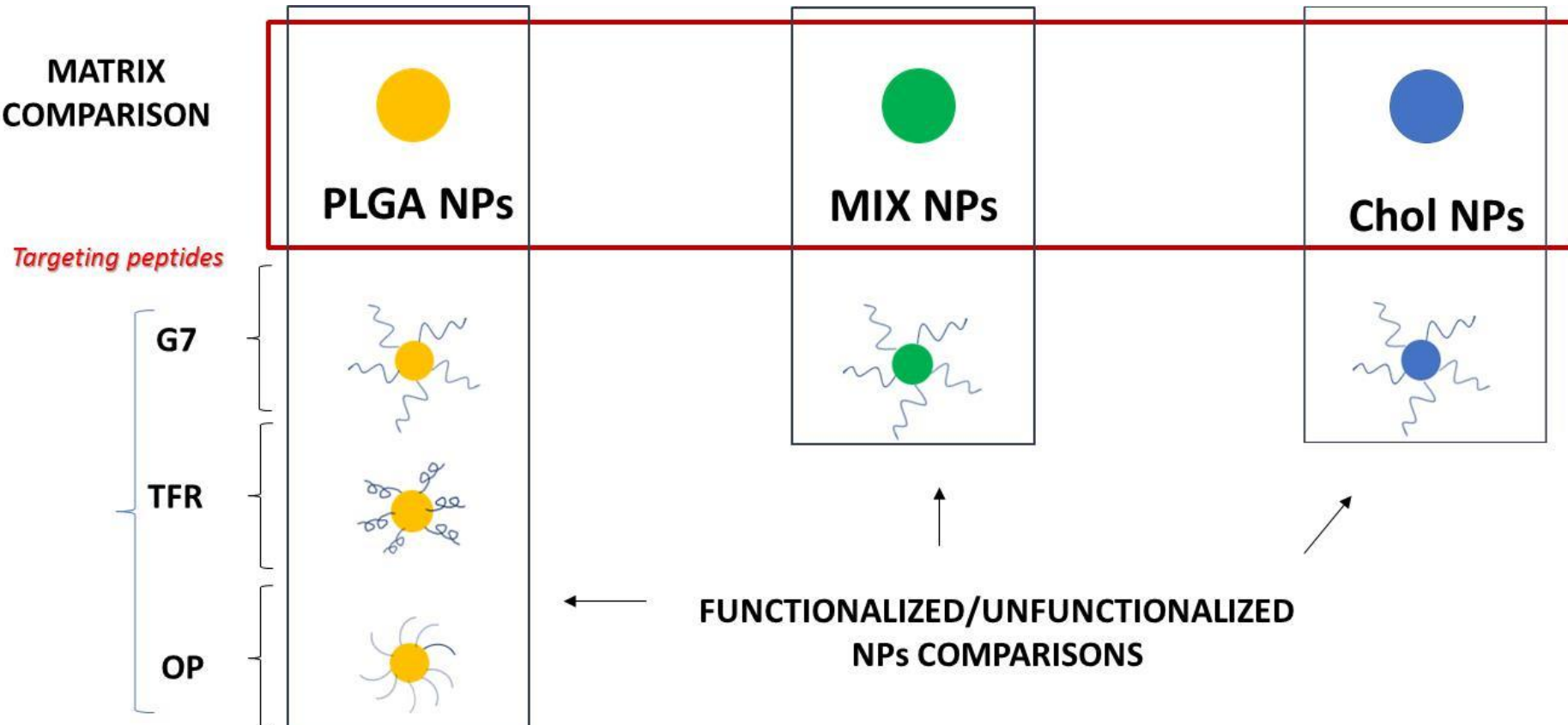
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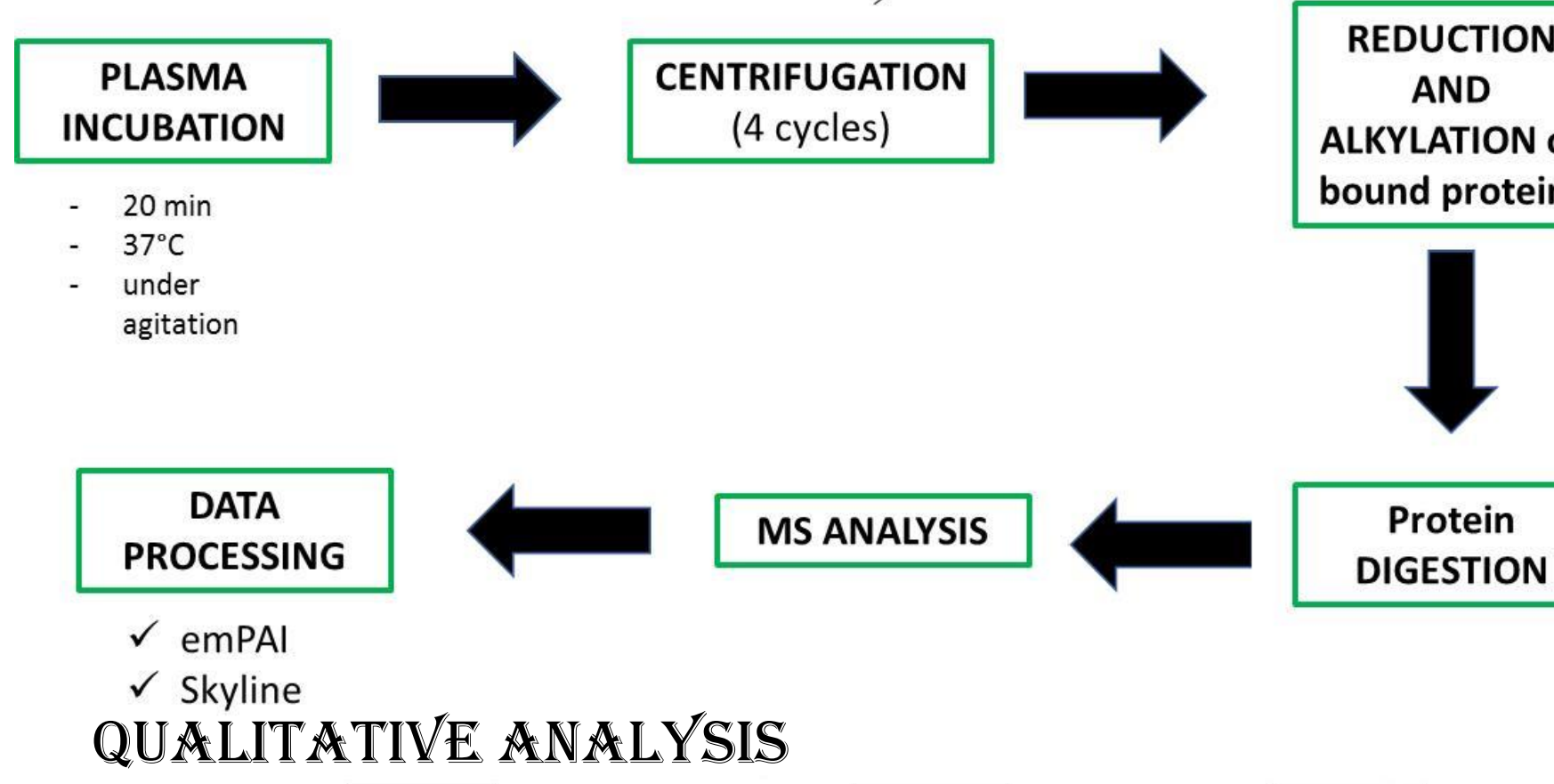
Nanoparticles (NPs) represent one of the most important tools in nanomedicine. When intravenously administered, NPs can generate a more or less stable Protein Corona (PC) that could impact NPs circulation, biodistribution, drug targeting, cellular uptake and toxicity [Pederzoli et al., Nanomedicine, 2018, 13, 407-422]. The plasma PC, is unique for each NP type and can be affected by shape, size, and surface properties as well as biological parameters and do not generally correlate with their relative abundances in the plasma. For this reason, the study of the PC is necessary to predict the biological identity of NPs containing the PC to achieve the desired biological and therapeutic effect.

Work aim is to characterize the PC (both HC and SC) of different NPs types (functionalized and un-functionalized) in order to identify the different protein pattern considering the NPs composition as the unique variable.



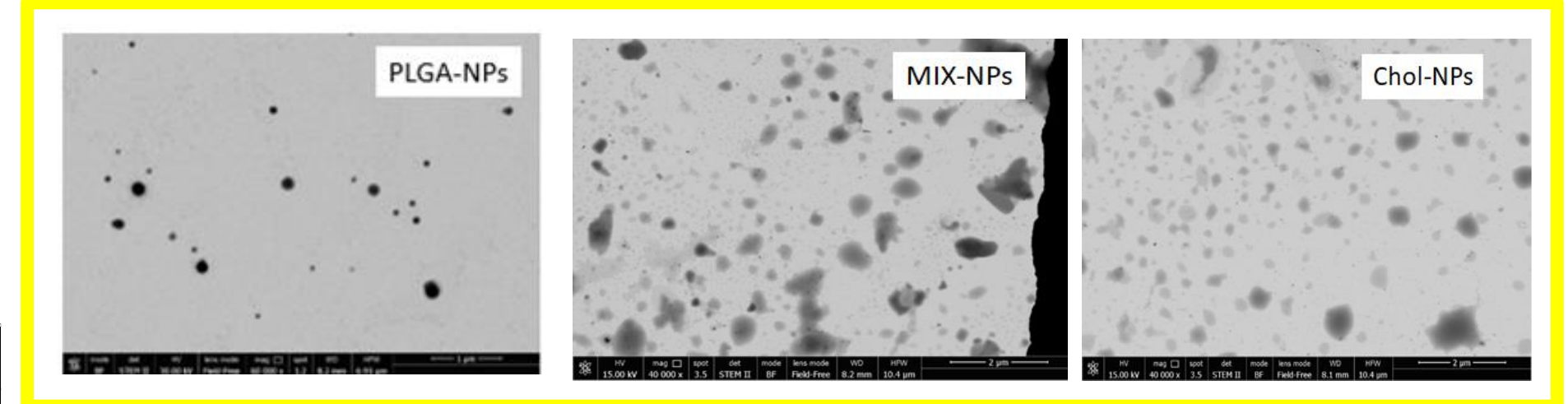
The PC was evaluated for 1) polymeric PLGA NPs, 2) hybrid NPs formulated with PLGA and cholesterol (MIX NPs) and 3) lipidic NPs (Chol NPs). Then, the same NPs were surface modified with well know brain targeting ligands each with different BBB crossing mechanisms; a) hepta-peptide G7 (15% of injected dose; membrane-membrane interaction and macropinosytosis-like mechanisms) 2) TFR peptide (2% of injected dose; transferrin receptor mediated crossing) and 3) a siml opioid peptide, OP-peptide (opioid receptor mediated crossing).

HC-APPROACH



CHARACTERIZATION OF NPS

NP samples	Size (nm)	PDI (nm)	Zpot (mV)	Weight yield (% w/w)	% (w/w) Pluronic
PLGA-NPs	152 (3)	0,12 (0,02)	-34,3 (6,2)	74,9 (6,6)	14 (1)
G7-PLGA NPs	150(3)	0,11 (0,01)	-33,3 (6,2)	84,3 (4,8)	14 (2)
TFR-PLGA NPs	164 (3)	0,15 (0,03)	-33,3 (6,4)	76,2 (-11,1)	12 (2)
OP-PLGA NPs	164 (5)	0,15 (0,02)	-29,2 (5,3)	82,5 (4,7)	16 (2)
MIX- NPs	237 (7)	0,26 (0,01)	-25,8 (5,3)	76,7(6,6)	27 (2)
G7-MIX-NPs	227 (5)	0,26 (0,02)	-23,2 (5,1)	76,2(5,7)	23 (3)
Chol-NPs	257 (14)	0,18 (0,05)	-19,1 (6,8)	78,7(6,0)	26 (9)
G7-Chol-NPs	234 (13)	0,25 (0,04)	-25,8 (3,6)	81,4(2,6)	26 (14)



Similar properties

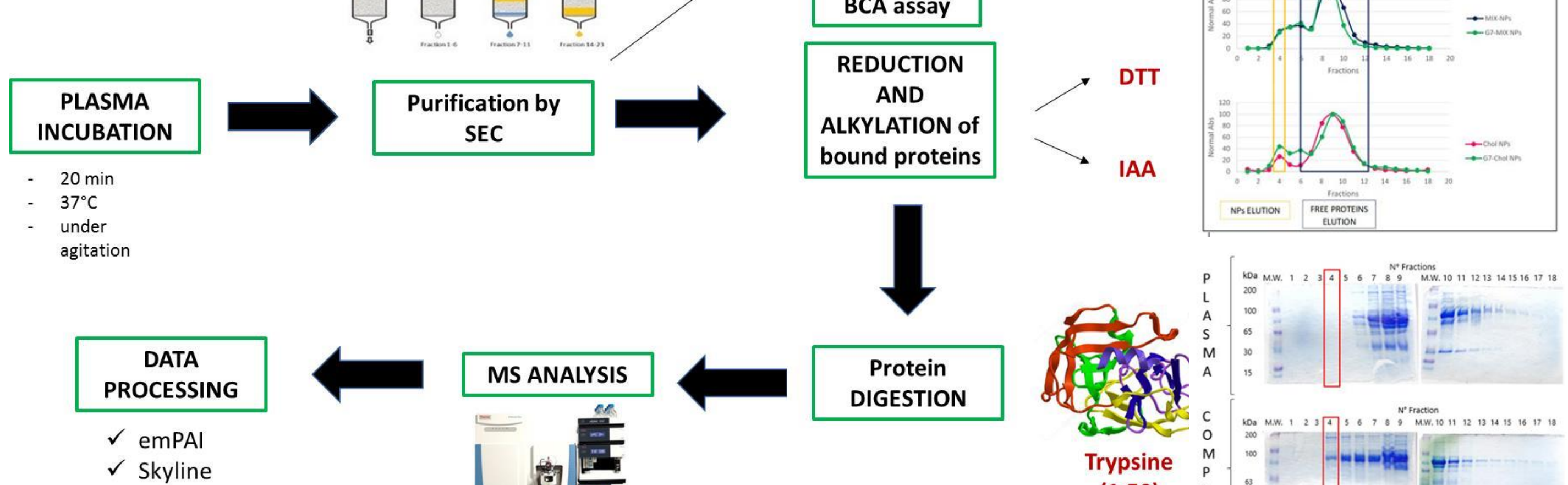
Similar properties

Similar properties

PERMITTED COMPARISONS:
-PLGA-based NPs -HYBRID NPs -
Chol-based NPs -HYBRID NPs vs
Chol-based NPs

Plasma concentration, incubation time and temperature, NPs concentration, NPs/plasma ratio are constant during analyses.

SC-APPROACH

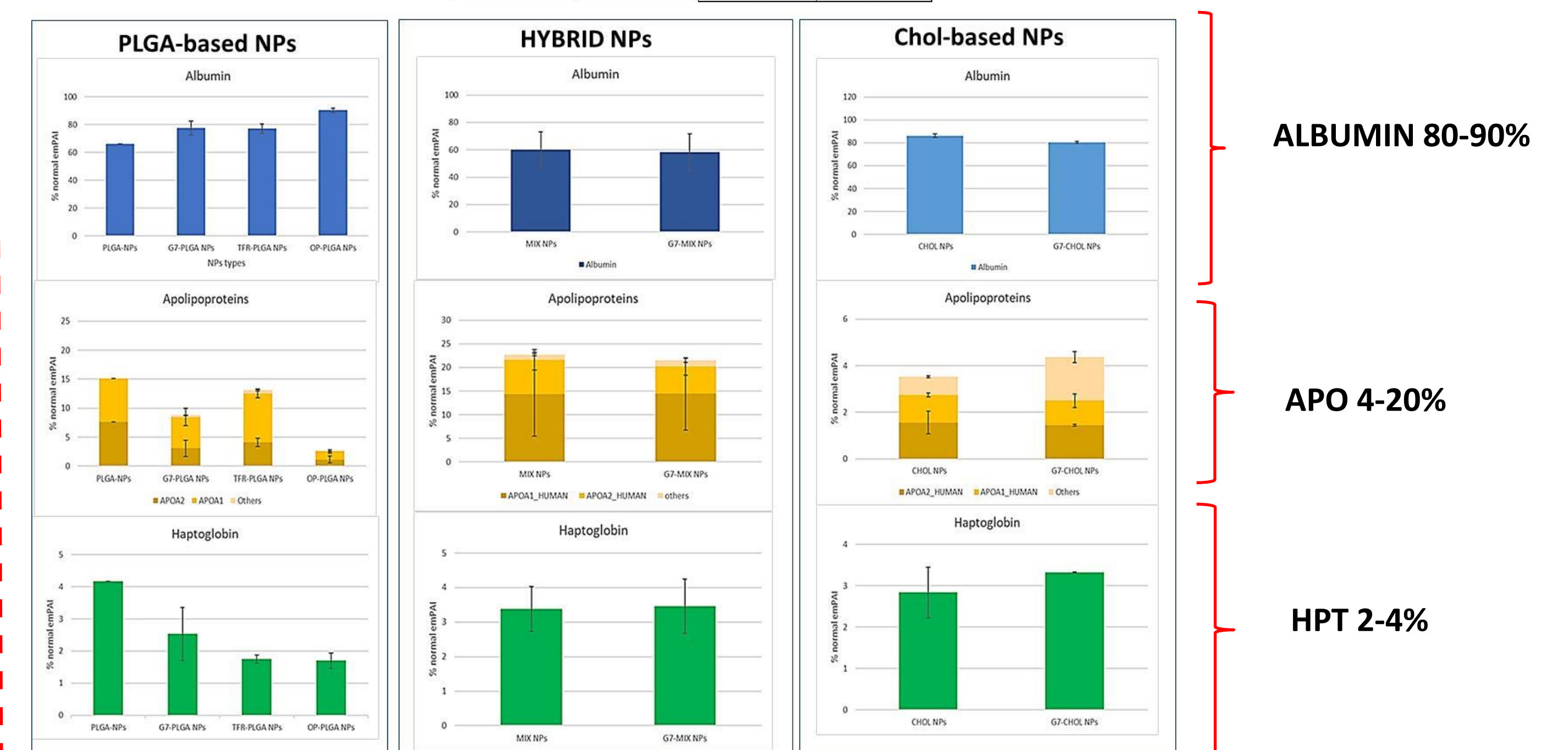
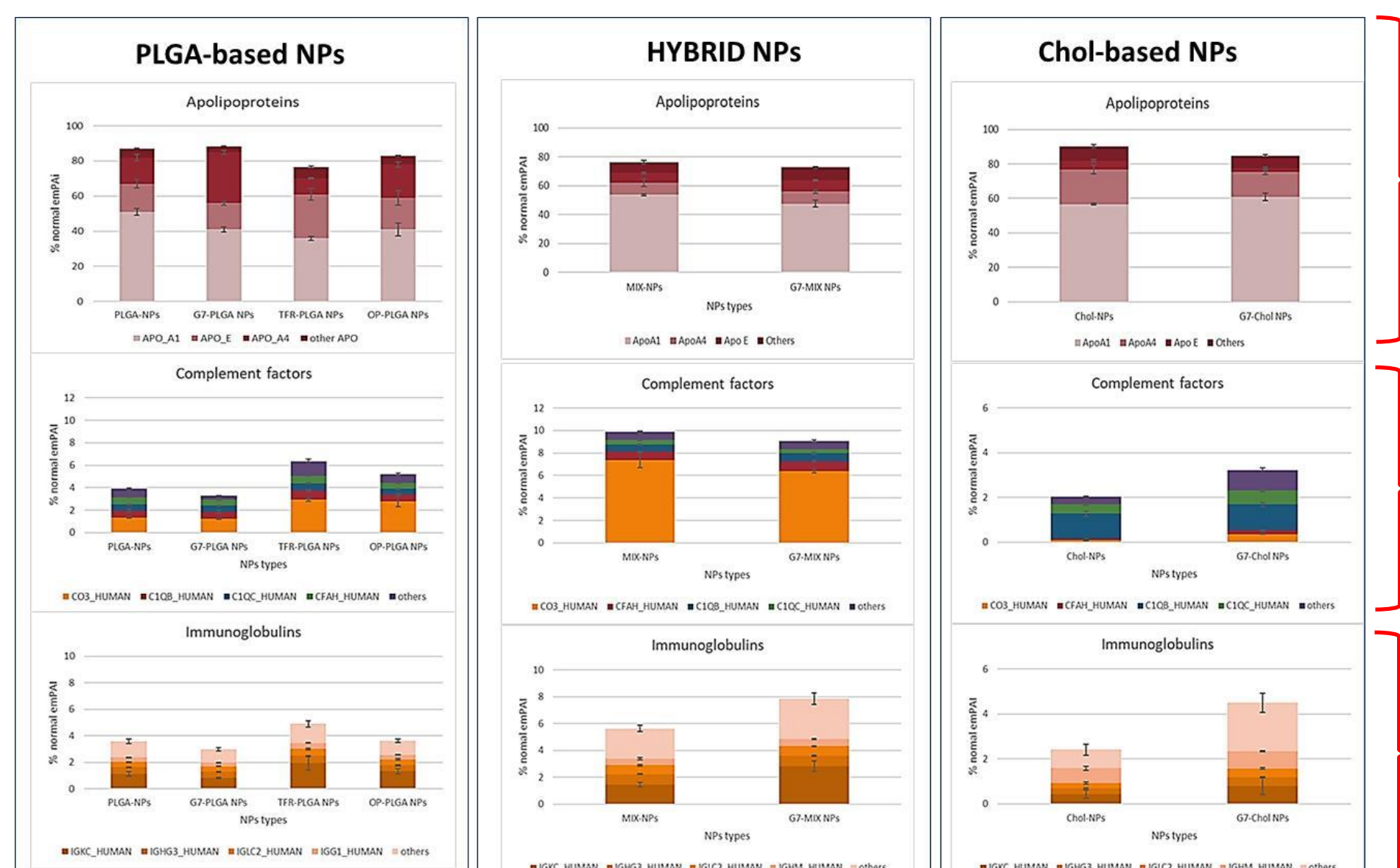


✓ HC is mainly composed with three predominant protein families (APO, COMPLEMENT FACTORS, IG)

✓ Higher content of immunogenic proteins in the HC of MIX-NPs (abundance of both immunoglobulins and complement factors.)

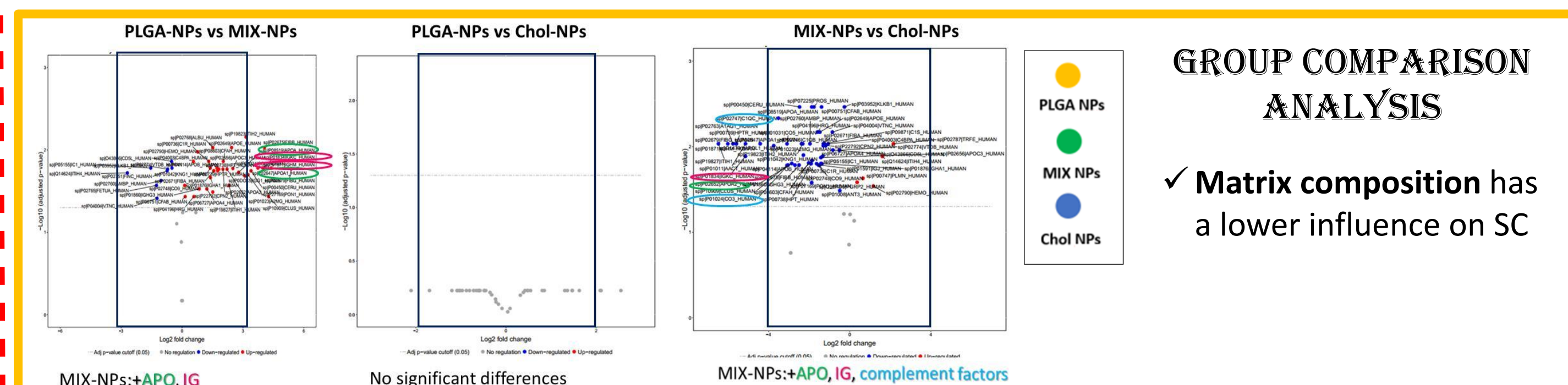
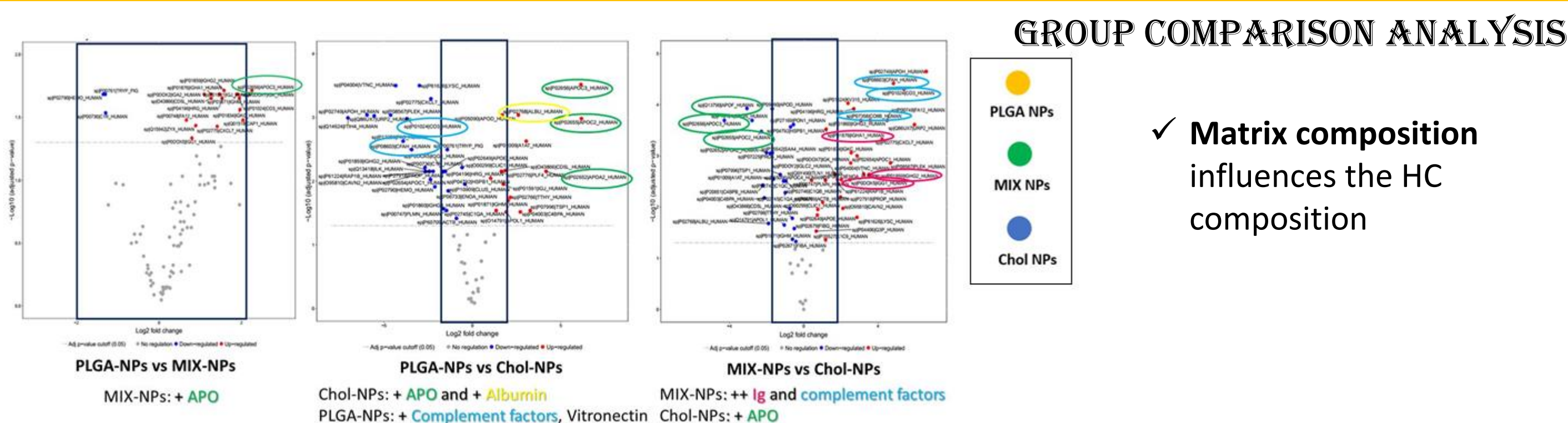
✓ The SC is mainly composed by ALBUMIN and other abundant plasma proteins (HTP, APO, IG and complement factors)

✓ MIX-NPs seem to absorb more apolipoproteins, complement factors and immunoglobulins respect to the other NP types.



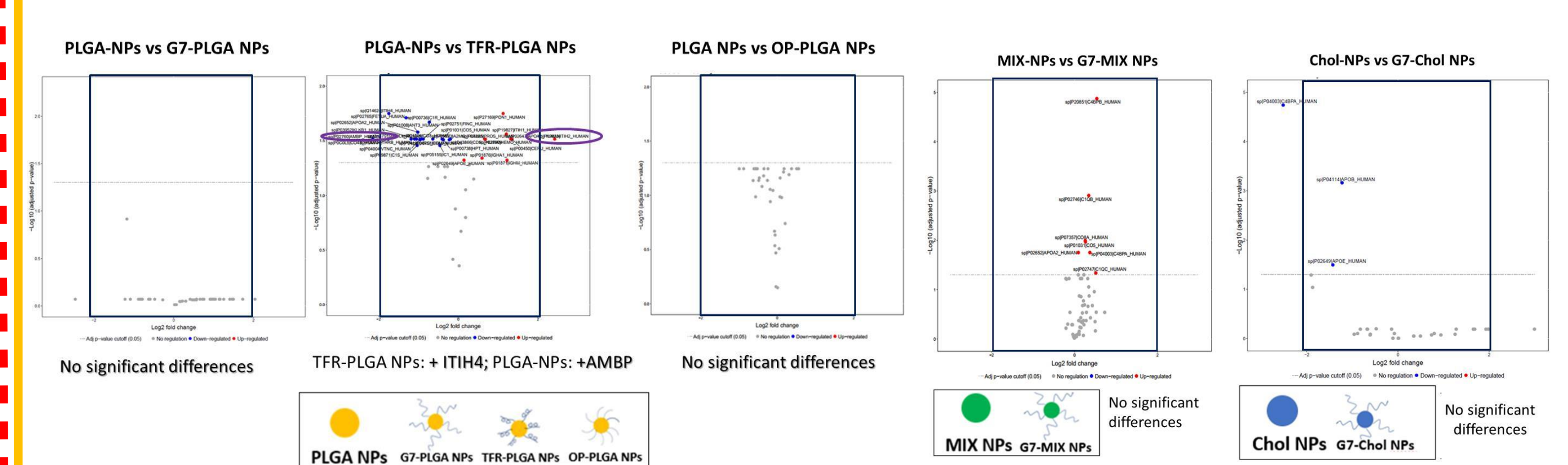
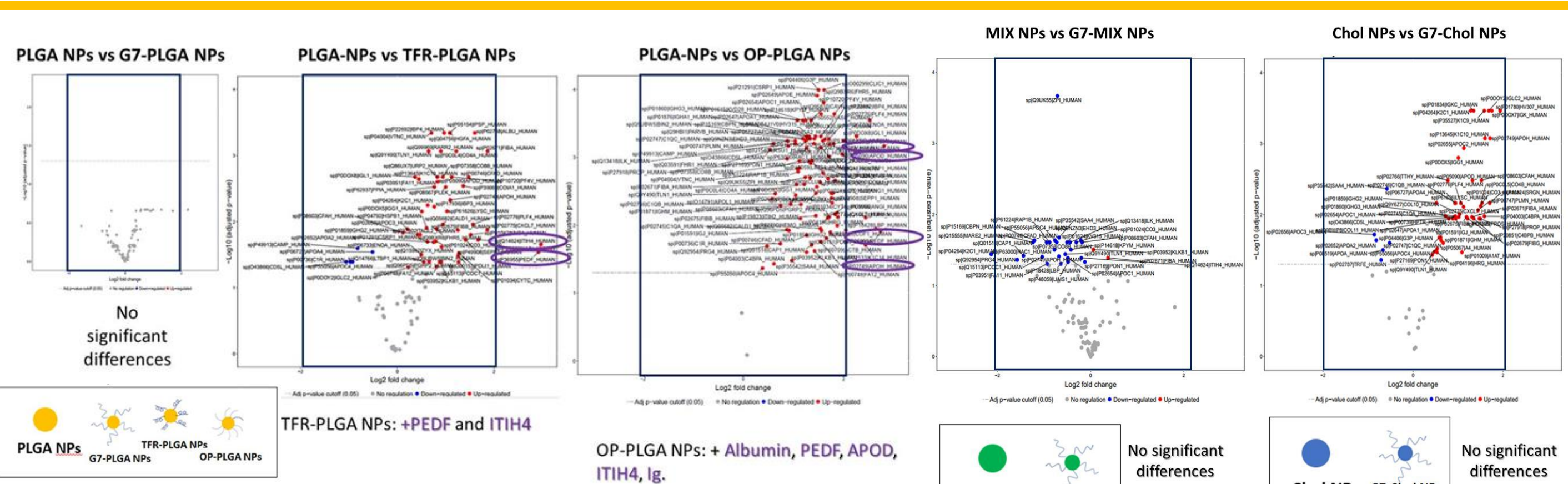
GROUP COMPARISON ANALYSIS

✓ Matrix composition influences the HC composition



GROUP COMPARISON ANALYSIS

✓ Matrix composition has a lower influence on SC



✓ No significant differences in the HC between bare and functionalized NPs



- Targeting ligands probably do not completely cover the NPs surface
- Unique targeting peptide: polymer ratio tested
- All different ligands are hydrophilic with similar chemical-physical features

✓ No significant differences in the PC between bare and functionalized NPs