

Processing of human proBNP in serum, EDTA-plasma and heparinized plasma: the matrix defines it all



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BACKGROUND: The paradox of high circulating levels of cardiac precursor to B-type natriuretic peptide (proBNP) with impaired activity in patients with heart failure is a continuing matter of debates. An intriguing idea is that proBNP₁₋₁₀₈ may undergo processing in circulation giving rise to active BNP₇₇₋₁₀₈. However, today the data regarding proBNP processing in circulation is limited. As an *in vitro* model systems for investigation of extracellular proBNP processing, serum and plasma have been suggested by several research groups.

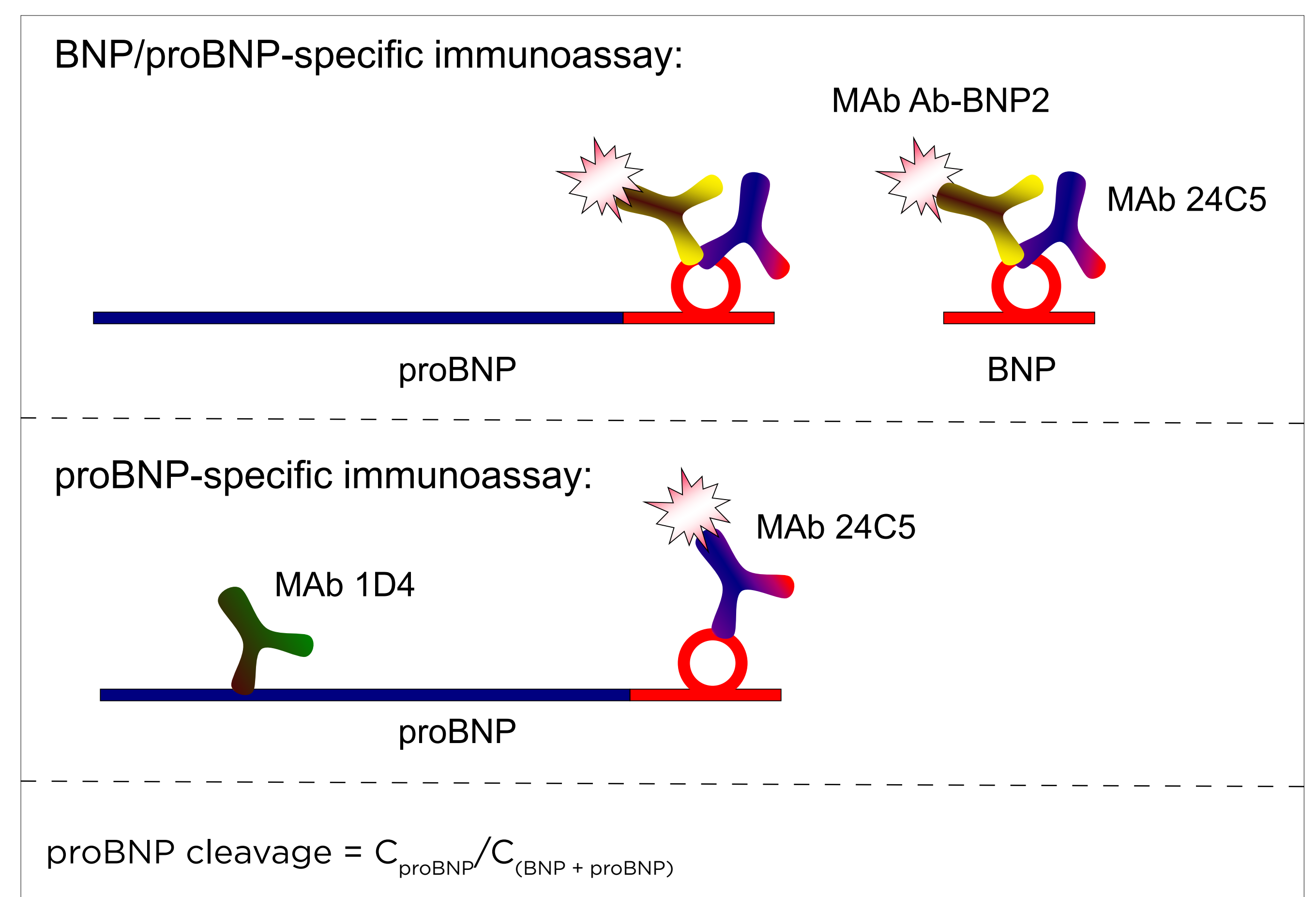
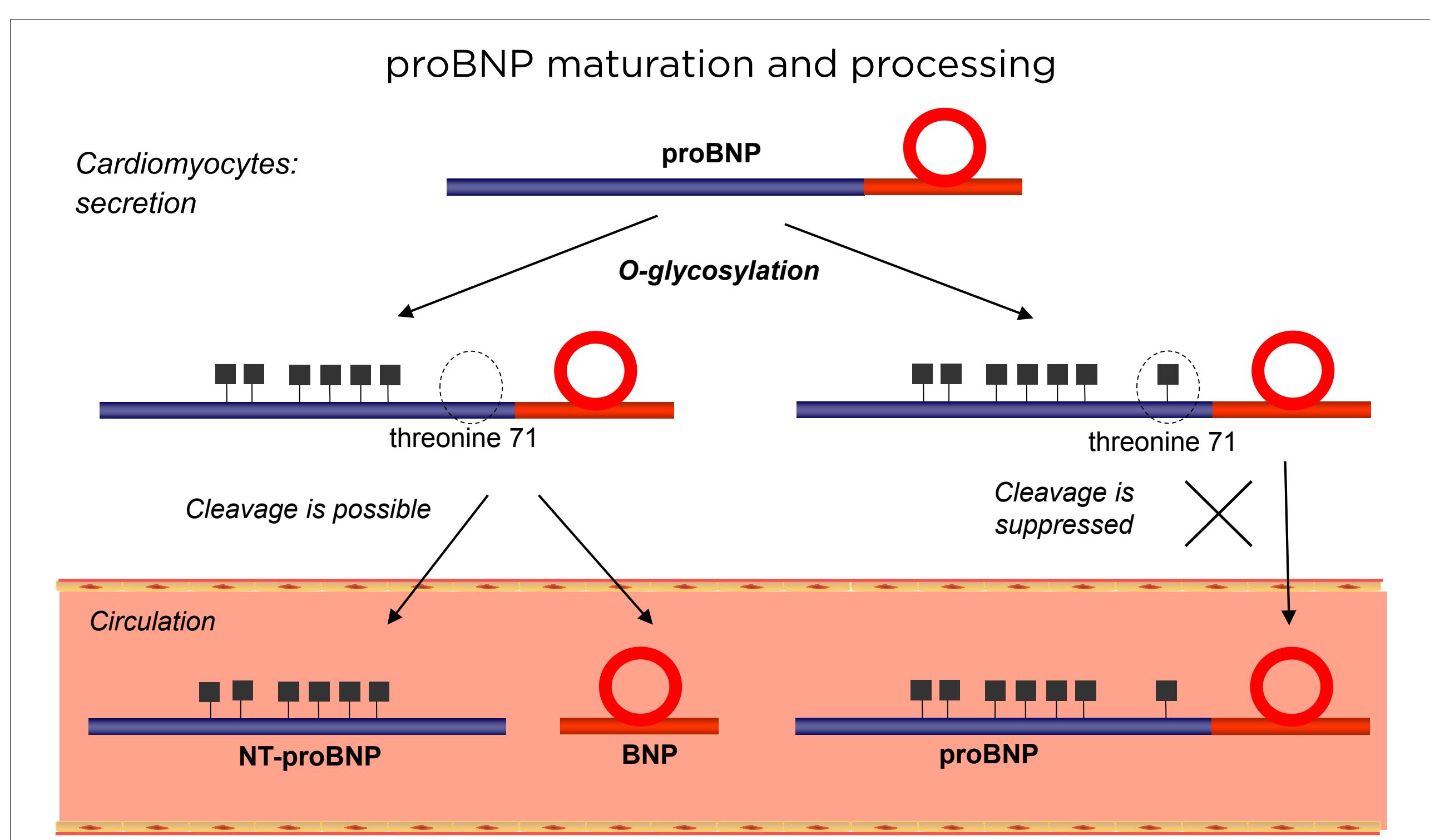
THE AIM OF THIS STUDY: to evaluate the relevance of serum and plasma as cell-free matrixes for studying proBNP processing in circulation.

Tools and Methods

MODEL SYSTEM: recombinant human non-glycosylated proBNP₁₋₁₀₈ (produced in *E. coli*, HyTest) was used in all the experiments as a proBNP form which is potentially susceptible to processing in appropriate conditions, since it has no attached glycan residues (see the scheme below).

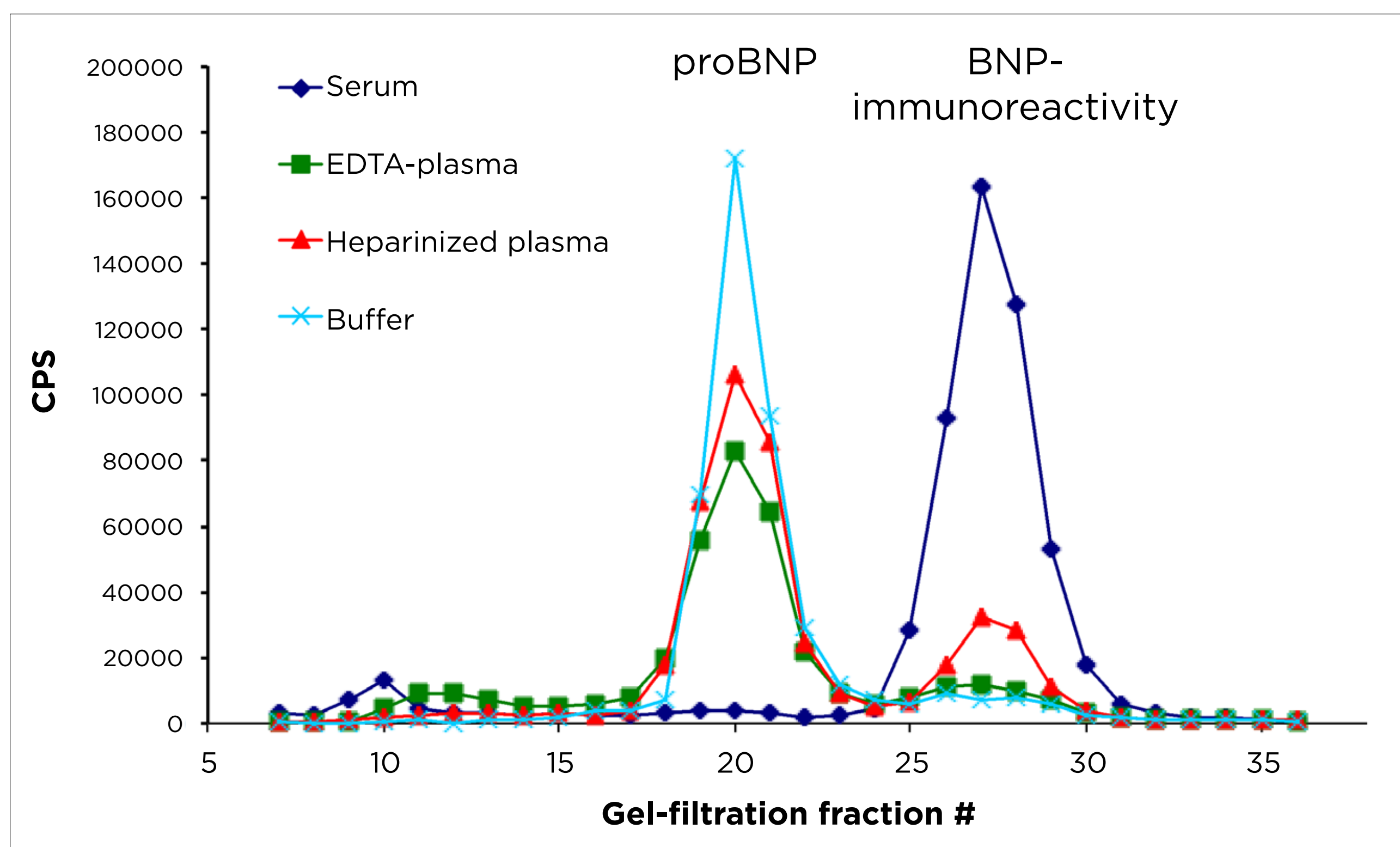
METHODS:

- Serum, EDTA-plasma or heparinized plasma samples of the same donor were tested for their ability to process recombinant proBNP.
- The cleavage of spiked proBNP was analyzed by means of gel-filtration (Superdex 75 column) combined with immunoassays specific to BNP/proBNP (antibodies from HyTest).
- MALDI-MS analysis was applied to assess BNP-related forms (extracted with anti-BNP sepharose) produced from spiked proBNP.
- A broad panel of protease inhibitors was tested to identify the type of proteolytic enzymes involved in proBNP cleavage: furin inhibitor I (Dec-RVKR-CMK), kallikrein inhibitor PPACK II (H-D-Phe-Phe-Arg-CMK), PMSF, AEBSF, leupeptin, poly-arginine, soybean trypsin inhibitor, benzamidin, EDTA and heparin.



Results and Discussion

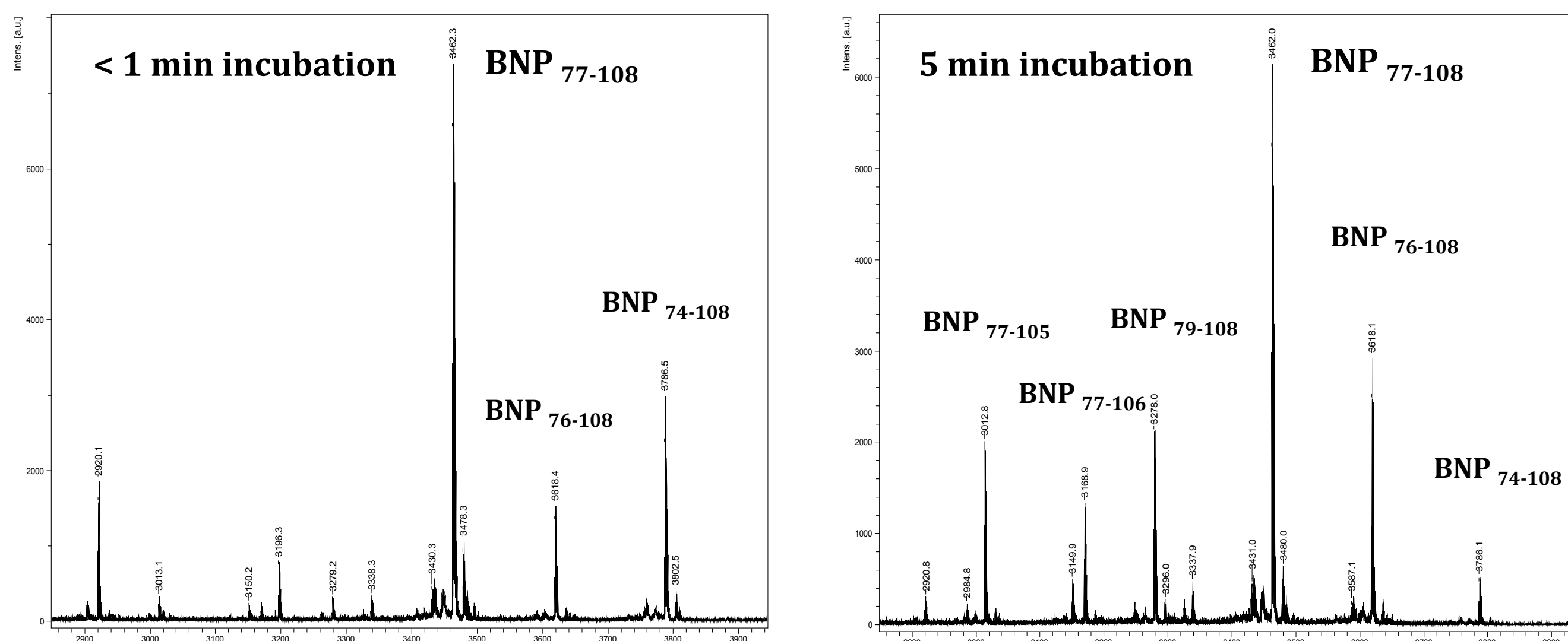
1. The efficiency of cleavage of spiked proBNP is matrix-dependent.



ProBNP is cleaved in serum (with the most prominent effect) and heparinized plasma, however, it stays almost intact in EDTA-plasma as follows from GF studies.

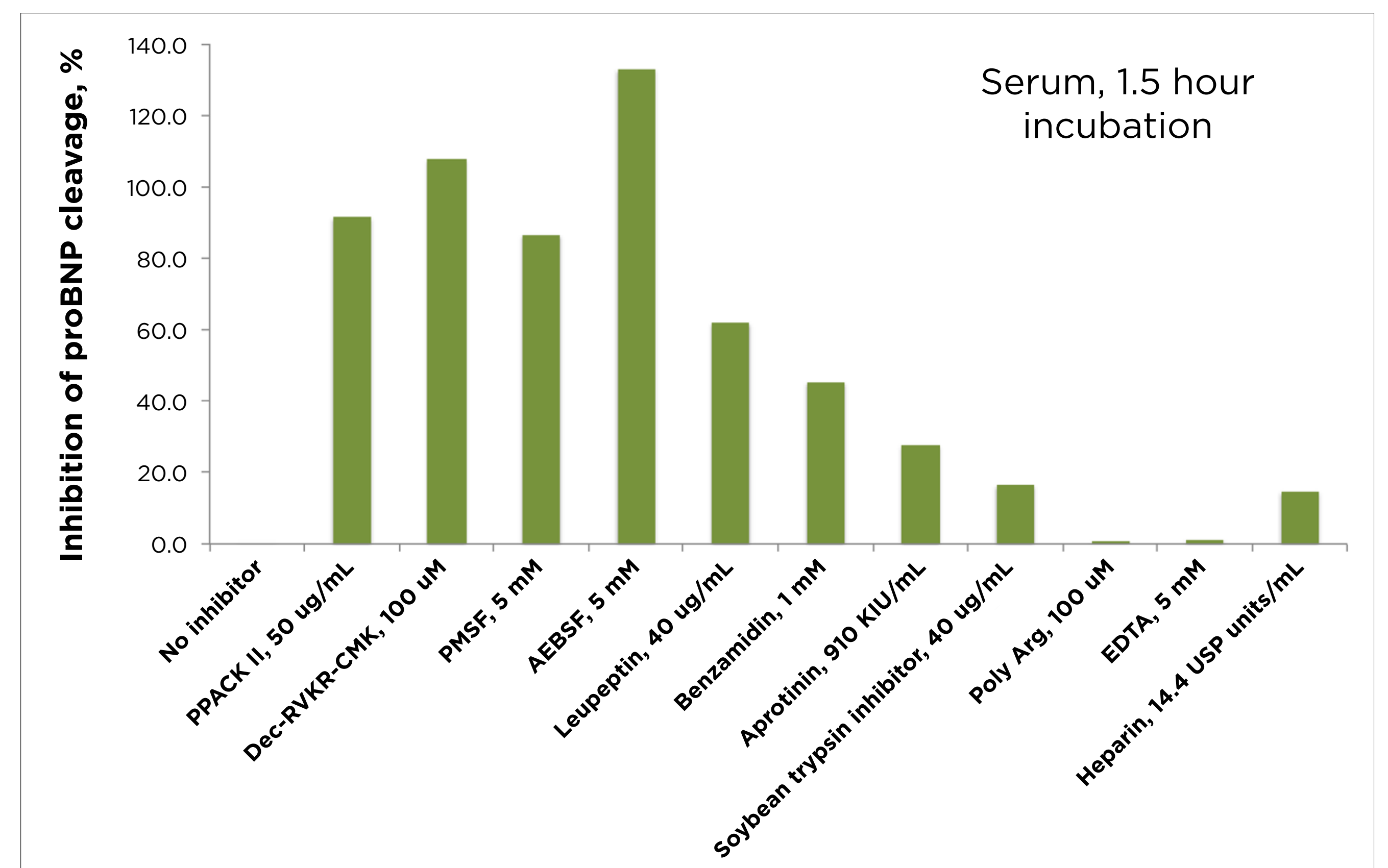
2. Cleavage of spiked proBNP in serum and heparinized plasma gives rise to longer than the canonical BNP₇₇₋₁₀₈ forms along with truncated forms.

MALDI-MS (non-quantitative)



Longer than canonical BNP₇₇₋₁₀₈ peptides are produced: BNP₇₄₋₁₀₈ and BNP₇₆₋₁₀₈. These longer BNP forms undergo degradation giving rise to shorter peptides: e.g. BNP₇₇₋₁₀₈, BNP₇₉₋₁₀₈, BNP₇₇₋₁₀₆, BNP₇₇₋₁₀₅ etc.

3. Inhibition of cleavage of spiked proBNP is observed under addition of broad range of inhibitors.



The inhibition profile of proBNP cleavage in serum and heparinized plasma can not be attributed to any known sole prohormone convertase.

Conclusions

- Cleavage of proBNP in serum and plasma exhibits different efficiency and gives rise to a variety of BNP-related forms, suggesting unspecificity of this process.
- A broad range of inhibitors are able to block cleavage of spiked proBNP, suggesting involvement of a group of proteases activated during blood sample matrices preparation.
- The present data show the limitations of serum and plasma as model systems to explore proBNP processing in circulation and highlight the importance of choosing relevant *in vivo* models instead of *in vitro* ones.