# Rules to prepare peptide-imprinted nanogels with high-affinity binding suitable for sensing and assays by precipitation polymerization

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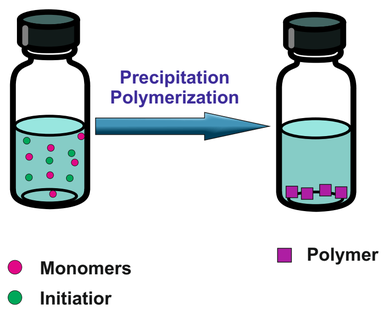
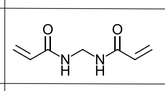
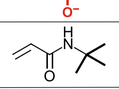
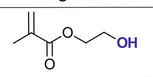
## INTRODUCTION

Molecular imprinting is a technique for preparing polymeric scaffolds (Molecularly Imprinted Polymers, MIPs) that act as synthetic receptors and show affinity and selectivity towards a target analyte [1]. When MIPs are downsized to the nanoscale (nanoMIPs), they show an increase in the number of accessible imprinted binding cavities per material weight and an enhanced molecular recognition ability, leading to faster binding kinetics, higher affinity and selectivity [2,3]. Being the recognition properties of the nanoMIPs strictly correlated to the effective formation of the imprints in the chosen synthetic conditions, a deeper comprehension of the polymerization at the nanoscale is required.
Here we present a study of the best conditions to form imprints at the nanoscale when the synthesis occurs by a precipitation polymerization protocol, using as target analyte the peptide of Troponin I, clinical marker of cardiac failure [4]. By exploring a range of monomers combinations, polyacrylamide-based MIP nanogels having homogeneous nano-dimensions and a low number of binding sites per nanoparticle were synthesized. To this purpose, we evaluated the influence of the monomer composition and the total monomers to template molar ratio on the hydrodynamic sizes and on the recognition properties, respectively, defining the conditions to tune the nanoMIP dimensions (from 60 to >600 nm) and to improve the efficacy of the imprinting process.

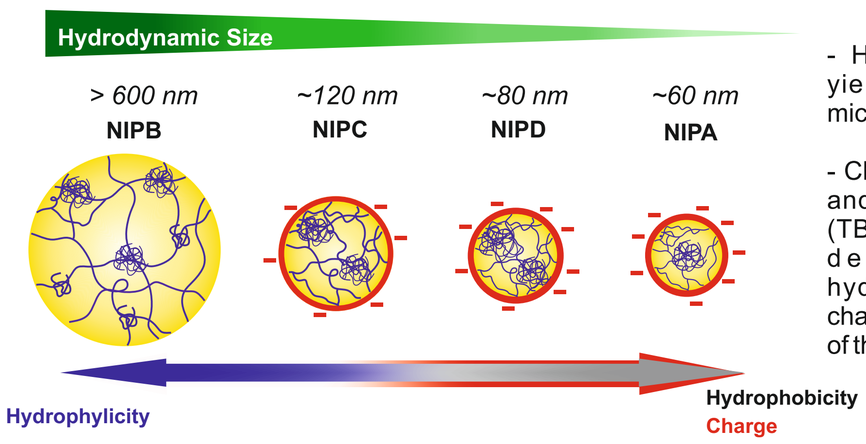
## CONTROL OF THE POLYMERIZATION AT THE NANOSCALE

### Nanogels synthesis at different monomers combination




| | | NIPB | NIPC | NIPD | NIPA |
|---|---|---|---|---|---|
| **BIS** (% mol) crosslinker |  | 80 | 80 | 80 | 80 |
| **Aam** (% mol) neutral acrylamide |  | -- | -- | -- | 8 |
| **MAA** (% mol) charge methacrylic acid |  | -- | 8 | 8 | 8 |
| **TBAm** (% mol) hydrophobic |  | -- | -- | 4 | 4 |
| **HEMA** (% mol) hydrophylic methacrylate |  | 20 | 12 | 8 | 0 |

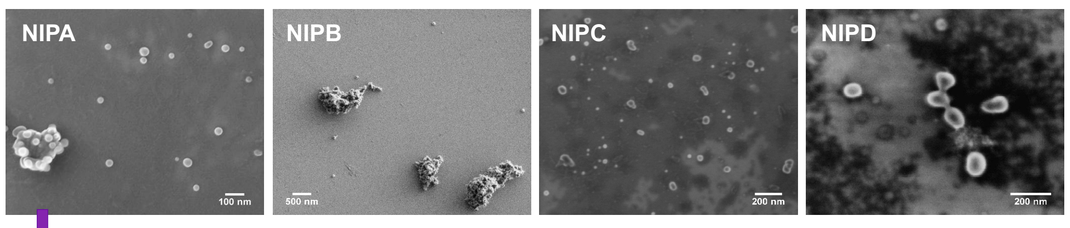
### Different packing of polymeric structures for the different compositions




- Hydrophilic monomers yield to sub-micron to micron-sized particles.

- Charge repulsion (MAA) and hydrophobic effect (TBAm) contribute to a denser packing of hydrophobic polymeric chains, limiting the growth of the nanogels.

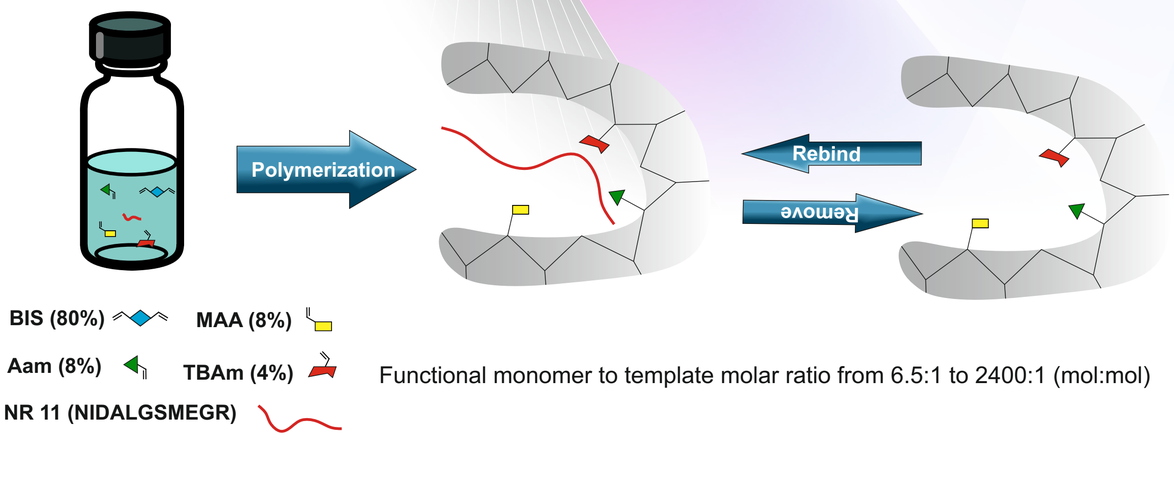
### Physical Characterization of dried nanoparticles




NIPA show the higher size homogeneity, collidal stability and the smaller dimensions

## IMPRINTED BINDING SITES IN NANOGELS

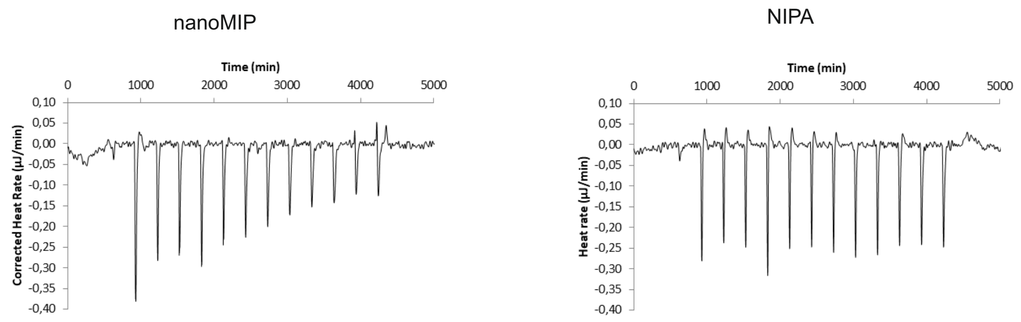
### nanoMIP synthesis using Troponin I peptide epitope NR11 as template



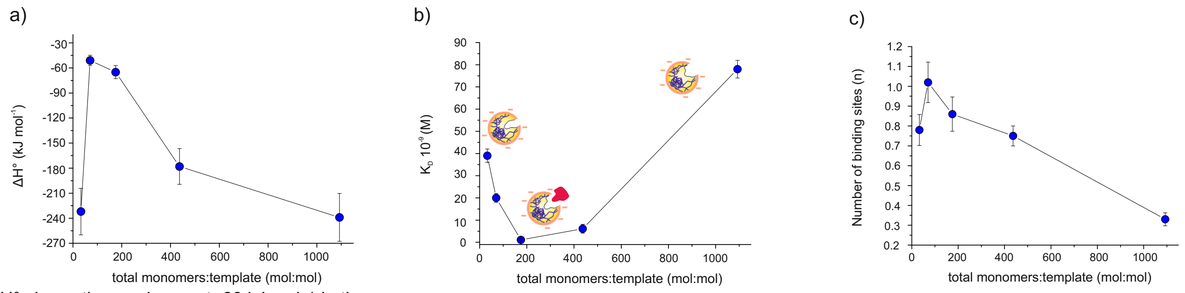

Functional monomer to template molar ratio from 6.5:1 to 2400:1 (mol:mol)

### Functional Characterization

The affinity of nanoMIP was measured by Isothermal Titration Calorimetry (ITC): nanogels (1.2 µM ) were titrated with NR11 (10 µM) and the heat exchange was measured. NIPA was used as negative control.




Raw data were intergated and the thermodynamic parameters (enthalpy ΔH°, dissociation constant Kd, number of binding sites n) were calculated with an independent site model (Langmuir).




ΔH° shows the maximum at -60 kJ mol-1 in the molar range 70≤TM:T≤175.
Being ΔH° typical value for reversible protein-protein interaction -20 and -60 kJ mol$^{-1}$ for antigen-antibody pairs, this let hypothesize extent of nanoMIP surface interacting with NR 11 to be similar to that of a protein binding site.

$K_D$ were nanomolar.
The curve shows a parabolic shape with minimum ($K_D$ ~$10^{-9}$ M) for the molar range 175≤TM:T≤437.
The $K_D$ minimum indicates the best ratios to get high affinity imprinted sites

Estimated number of high affinity binding sites, n, is approximatly one per nanoMIP particle.

## CONCLUSIONS

With the aim to define general rules for the preparation of nanoMIPs having homogeneous nano-dimensions and a low number of specific binding sites per nanoparticle, we studied the polymerization of nanoMIPs in precipitation conditions in aqueous solution when imprinted with a peptide (NR11).
Playing with a set of acrylamide-based and methacrylate monomers, well compatible with protein and peptide templates, we demonstrated how the monomer composition determines the size of the resulting material, so to custom-prepare both micro- and nanogels. Charge repulsion and hydrophobic effect contributed at most to the formation of the nanoMIPs (~60 nm) whereas hydrophilic monomers yielded to sub-micron- to micron-sized particles.
The efficacy of the stamping events in the chosen polymerization conditions resulted maximal for TM:T molar ratios encompassing 175:1 to 437:1. Within these conditions the nanoMIPs showed nanomolar dissociation constants for the template and a mean number of one binding site per nanogel particle.
The present results contribute to define rules to imprint peptides at the nanoscale and have a practical impact on the production of synthetic recognition materials.

## REFERENCES

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