Guanidinium Salts Can both Cause and Prevent the Hydrophobic Collapse of Biomacromolecules

Jan Heyda\textsuperscript{1,2}, Halil Okur\textsuperscript{3}, Jana Paterova\textsuperscript{5}, Joachim Dzubiella\textsuperscript{2,4}, Pavel Jungwirth\textsuperscript{5}, Paul Cremer\textsuperscript{3,6}

\textsuperscript{1} Physical Chemistry Department, UCT Prague, Czech Republic
\textsuperscript{2} Institut für Weiche Materie und Funktionale Materialien, HZB Berlin, Germany
\textsuperscript{3} Chemistry Department, Penn. State University, Pennsylvania, USA
\textsuperscript{4} Institut für Physik, HU Berlin, Germany
\textsuperscript{5} Institute of Organic Chemistry and Biochemistry, CAS, Prague, Czech Republic
\textsuperscript{6} Biochemistry and Molecular Biology Department, Penn. State University, USA

DPG Spring meeting, Dresden, 2017
It is generally accepted that:

- binding additives induce denaturation (i.e., prefer states with larger surface)
- excluded additives induce stabilization (less exclusion from the smaller surface)

**Guanidinium** cation is one of the most powerful protein denaturants.

However, its effect depends on the counter anion (according to Hofmeister series of ions)

- Stabilizing: $\text{Gnd}_2\text{SO}_4 < \text{NaCl} < \text{sugar} < \text{water} \ll \text{GndCl} \ll \text{GndSCN}$

Street, Bolen, Rose, PNAS 103 (2006) 13997-14002
Rocco et al., Biophysical Journal 94 (2008) 2241-2251
Thermal stability of neutral ELP (cloud point measurements)

ELP = elastine = monodisperse thermoresponsive polypeptide (VPGVG)_{120}

(1M salt)

GndCl

+ Gnd2SO4

GndSCN

\[
\text{Salt Concentration [M]}
\]

\[
\text{LCST [°C]}
\]

30, 53 °C  PNIPAM_NaSCN

SRS OptiMelt 1
Thermal stability of neutral ELP (cloud point measurements)

ELP = elastine = monodisperse thermoresponsive polypeptide (VPGVG)_{120}

Why does GndSCN decrease LCST?
Is the action of GndSCN and Gnd\textsubscript{2}SO\textsubscript{4} similar?

Why does GndSCN decrease LCST?

Is the action of GndSCN and Gnd\textsubscript{2}SO\textsubscript{4} similar?
Thermal stability of neutral ELP (cloud point measurements)

ELP = elastine = monodisperse thermoresponsive polypeptide (VPGVG)_{120}

What is the origin of the nonlinearity in GndSCN?
Microscopic insight – ATR-FTIR spectroscopy

- Substantial enrichment of Gnd\(^+\) and SCN\(^-\) in the collapsed polymer
- Mild, resp. strong depletion of Gnd\(^+\) and SO\(_4\)\(^{2-}\)
Microscopic insight – ATR-FTIR spectroscopy

- Substantial enrichment of Gnd$^+$ and SCN$^-$ in the collapsed polymer
- Mild, resp. strong depletion of Gnd$^+$ and SO$_4^{2-}$
- Mild enrichment of Gnd$^+$/Cl$^-$
Microscopic insight – atomistic MD simulations

(A) • $\text{Gnd}_2\text{SO}_4$ is depleted (A)
• $\text{GndCl}$ is weakly attracted (B)
• $\text{GndSCN}$ is in a big excess (C)

(B) • Order to ELP-salt interaction strengths
$\text{GndSCN} \gg \text{GndCl} > \text{Gnd}_2\text{SO}_4$
In agreement with ATR-FTIR :-) 

(C) • Only affinity to extended chain can be studied in all-atom MD

• But, this three regimes of interaction strengths were employed on a generic coarse-grained level\(^1\)

\(^1\) Heyda, Muzdalo, Dzubiella, Macromolecules, 2013, 46, 1231
Generic coarse-grained simulations

- Employing LJ-beads-on-a-spring model of the polymer (100 beads)
- Varying single parameter – **salt-monomer interaction** – 3 qualitatively different scenarios are obtained

1) **Weak binding leads to gradual swelling** (GndCl)
2) **Depletion induced compression of the globule** (Gnd$_2$SO$_4$)
3) **Strong binding leads to rapid collapse and subsequent swelling** (GndSCN)

How? Chain cross-linking at low and maximization of interactions at high concentration.

Unified description within Kirkwood-Buff theory = preferential binding concept

Heyda, Muzdalo, Dzubiella, Macromolecules, 2013, 46, 1231
Conclusion

- Unlike most of the cations, Gnd$^+$ is enriched in the polymer vicinity.
- The enrichment of Gnd$^+$ is modulated by the counterion:
  - strongly depleted SO$_4^{2-}$
  - weakly attracted Cl$^-$
  - strongly attracted SCN$^-$
- Weak binding of GndCl leads to gradual solubilization of ELP.
- Depleted Gnd$_2$SO$_4$ salts ELP out of the solution.
- Strong binding of GndSCN induces:
  - at low concentrations $\rightarrow$ globular polymer states
    (crosslinking of the polymer chains)
  - at high concentrations $\rightarrow$ coil polymer states
    (maximizing surface for interactions)
- This effect GndSCN is not observed in proteins
  - due to unique native fold
  - but the ensembles of denatured states should be very different in GndCl and GndSCN.
- The Coil $\rightarrow$ Globule $\rightarrow$ Coil process resembles the cononsolvency effect (when mixture of two good solvents becomes bad).

Guanidinium can both Cause and Prevent the Hydrophobic Collapse of Biomacromolecules

Jan Heyda,*†,‡ Halil I. Okur,‖ Jana Hladílková,⊥,⊗ Kelvin B. Rembert,‖ William Hunn,§ Tinglu Yang,‖ Joachim Dzubiella,†,#,¶ Pavel Jungwirth,*,⊗ and Paul S. Cremer*,‖,∥,△

ABSTRACT: A combination of Fourier transform infrared and phase transition measurements as well as molecular computer simulations, and thermodynamic modeling were performed to probe the mechanisms by which guanidinium (Gnd⁺) salts influence the stability of the collapsed versus uncollapsed state of an elastin-like polypeptide (ELP), an uncharged thermoresponsive polymer. We found that the cation’s action was highly dependent upon the counteranion with which it was paired. Specifically, Gnd⁺ was depleted from the ELP/water interface and was found to stabilize the collapsed state of the macromolecule when paired with well-hydrated anions such as SO₄²⁻. Stabilization in this case occurred via an excluded volume (or depletion) effect, whereby SO₄²⁻ was strongly partitioned away from the ELP/water interface. Intriguingly, at low salt concentrations, Gnd⁺ was also found to stabilize the collapsed state of the ELP when paired with SCN⁻, which is a strong binder for the ELP. In this case, the anion and cation were both found to be enriched in the collapsed state of the polymer. The collapsed state was favored because the Gnd⁺ cross-linked the polymer chains together.
Acknowledgement

- Pavel Jungwirth (IOCB Prague)
- Joachim Dzubiella (HZB&HU Berlin)
- Paul Cremer (Pennsylvania State University) and students, postdocs, and collaborators

Funding:
- GAČR (2016-2019) - Microscopic insight into collapse thermodynamics of thermoresponsive polymers (16-24321Y)
- Alexander von Humboldt foundation (fellow 2012-14), (Czech install. 2015-16)
Acknowledgement

- Pavel Jungwirth (IOCB Prague)
- Joachim Dzubiella (HZB&HU Berlin)
- Paul Cremer (Pennsylvania State University)
- and students, postdocs, and collaborators

Thank you for your attention!

Funding:
- GAČR (2016-2019) - Microscopic insight into collapse thermodynamics of thermoresponsive polymers (16-24321Y)
- Alexander von Humboldt foundation (fellow 2012-14), (Czech install. 2015-16)