Supplementary Data S1: Protein and DNA extraction profiles from urinary pellet (UP) samples associated with UTIs

Page 2 (Figure 1). SDS-PAGE analysis of urinary pellet (UP) lysates including aggregate-forming UP (AUP) samples and easily dispersed UP (DUP) samples

The lysates were generated by suspension of an aliquot of a urinary pellet in a 1% SDS solution containing 10 mM Na-EDTA and 20 mM DTT. Samples were boiled at 95°C for 5 min, cooled to room temperature and spun at 16,100 x g for 5 min to remove any unsolubilized matter. The SDS-PAGE gels (4-25% NuPAGE gels) were stained with Coomassie Brilliant Blue G250 to visualize the proteins. The sample identifier (sample ID) is used throughout the Supplementary Data S1 and the main manuscript.

Pages 3-22 (Figures 2a to 2t). DNA and protein extraction profiles from UP samples with evidence of aggregation and protein solubilization during a DNAse I incubation step - suggesting NET formation - and control UP samples that did not form aggregates

Page 23 (Figure 3). Western blot confirming the reactivity of S. aureus protein A (Spa) with anti-rabbit IgG HRP conjugate

General descriptions for the data in Figures 2A to 2R:
This dataset pertains to the extraction of urinary pellet (UP) samples that were visually inspected for physical appearance and aggregation behavior. We examined aggregate-forming UP (AUP) samples. Such aggregates rich in neutrophils formed upon re-suspension of the urinary pellets in PBS or in PBS supplemented with dithiothreitol (DTT). We also examined a few UP samples that were not aggregating in the process of resuspension and extraction with PBS-DTT. The pellets remained dispersed after re-suspension (DUP samples). The extractions for one UP sample was repeated (#134 in this dataset). Freeze-thaw cycles caused contributed to the aggregation of cellular and extracellular matter in the AUP samples.

The first AUP sample (#33) on Page 3 contains extensive information of the fractionation methods and observations related to many AUP samples. The DUP samples (as controls) were from clean catch urine pellets with relatively low neutrophil counts (#9; #29; #36; #55; #88) and one sample derived from a catheter biofilm (#64): Pages 4 to 9. I many but not all cases, a DNA agarose gel was run together with an SDS-PAGE gel to visualize the released DNA in the isolated fractions.

Please note that the sample identifiers (IDs) are the same on Page 1 (UP lysates) and on Pages 3-22 (fractions of the lysates).
$P_\text{id}$, species identified by LC-MS/MS and microbial culture

Gv, *Gardnerella vaginalis*

Bf, *Bacteroides fragilis*

Kp, *Klebsiella pneumoniae*

Pm, *Proteus mirabilis*

Ec, *Escherichia coli*

Sa, *Staphylococcus aureus*

Ef, *Enterococcus faecalis*

Ca, *Candida albicans*

Sm, *Serratia marcescens*

ND, not determined

(please note that these were the dominant microbial organisms; a few samples had evidence of up to three pathogens / commensals of the urogenital tract)

NC: leukocyte (neutrophil) counts assessed by standard urine sediment microscopy with a hemocytometer per high-power field

O2: more than 2 per HPF

O6: more than 2 per HPF

O11: more than 2 per HPF

tntc: leukocytes too numerous to count
NutPAGE gel, 4-12% acrylamide, Coomassie Brilliant Blue G250 stained
AUP sample 33

1: UPsol1, ca. 11 ul extract
2: UPsol2a, ca. 11 ul extract
3: UPsol2b, ca. 11 ul extract
4: UPsol3, ca. 11 ul extract
5: UPsol4, ca. 11 ul extract
6: UPsol5, ca. 11 ul extract
7: Mr standard, 5 ul solution

- Sample 33 was viscous and clumpy. *In vitro* culture and proteomic analysis revealed *Enterococcus faecalis* as the infectious agent. The extractions related to this experiment were performed after a freeze/thaw cycle. The sample 33 remained clumpy and viscous and was not homogenized upon incubation with PBS and PBS + 50 mM DTT (UPsol1 and UPsol2 fractions, respectively). The pellet derived from the UPsol2 extraction step was incubated with deoxyribonuclease I (DNAse I) for 60 min, resulting in the fraction UPsol3.
- Incubation steps with 50 µg/ml mutanolysin and 100 µg/ml lysosome in PBS or TBS followed and 10 mM EDTA and 0.4% CHAPS were added. Samples were vortexed, left at 20°C for 10 min, and sonicated at the amplitude 6 in ten 30 sec on/off cycles using a Misonex 3000 sonicator while cooling on ice. Following centrifugation for 6 min at 8,000 x g, a supernatant termed UPsol4 was isolated. The insoluble pellet was incubated with the SED solution (1% SDS, 0.3% Tween-20, 10 mM EDTA, 25 mM DTT) for 30 min followed by sonication at the amplitude 6 for 5 min (Misonex 3000 sonicator). The homogenate was vortexed, left at 20°C for 10 min, and heat-denatured at 95°C for 3 min. This was followed by repeated sonication step and centrifugation at 16,100 x g for 10 min to isolate the fraction UPsol5.

**Summary:**
Step 1: extraction with PBS (UPsol1);
Step 2: two successive extraction steps with PBS + 50 mM DTT (UPsol2a and UPsol2b);
Step 3: incubation/extraction with PBS and DNAse I (UPsol3);
Step 4: extraction/incubation with lysozyme and mutanolysin in the presence of the partially membrane-solubilizing reagents EDTA and CHAPS (UPsol4);
Step 5: incubation/heat extraction with denaturing solution (UPsol5);

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**Figure 2a**

| Lane | Description |
|------|-------------|
| 1    | Mr standard, 5 ul solution |
| 2    | UPsol1, ca. 11 ul extract |
| 3    | UPsol2a, ca. 11 ul extract |
| 4    | UPsol3, ca. 11 ul extract |
| 5    | UPsol4, ca. 11 ul extract |
| 6    | UPsol5, ca. 11 ul extract |
| 7    | Mr standard, 5 ul solution |

LTF: lactotransferrin
MPO: myeloperoxidase
DEFA1: neutrophil α-defensin 1

(abbreviations also used in other images)
Date: 04-22-16
NuPAGE gel, 4-12% acrylamide, Coomassie Brilliant Blue G250 stained

DUP sample 9

1: UP$_{sol}$1, ca. 10 ul extract
2: UP$_{sol}$2, ca. 10 ul extract
3: UP$_{sol}$3, ca. 10 ul extract
4: UP$_{sol}$5, ca. 10 ul extract
5: Mr standard, 5 ul solution

- Sample 9 was neither aggregated nor clumpy after resuspension in PBS. Proteomic analysis revealed presence of *Gardnerella vaginalis* with no evidence that the bacterium caused a urinary tract infection although some neutrophils were microscopically identified. The extraction experiments shown here were performed after a freeze/thaw cycle. The pellet of sample 9 was homogenized upon incubation with PBS and PBS + 50 mM DTT (UP$_{sol}$1 and UP$_{sol}$2 fractions, respectively). The pellet derived from the UP$_{sol}$2 extraction step was small and was incubated with DNase I for 60 min, resulting in the fraction UP$_{sol}$3.
- In this experiment, the following extraction steps were identical to those described in Figure 1A, with the exception that the extraction step resulting in UP$_{sol}$4 was not performed.

Summary:
Step 1: extraction with PBS (UP$_{sol}$1);
Step 2: extraction with PBS + 50 mM DTT (UP$_{sol}$2);
Step 3: incubation/extraction with PBS and DNase I (UP$_{sol}$3);
Step 4: incubation/heat extraction with denaturing solution (UP$_{sol}$5);
Date: 12-17-13

**NuPAGE gel, 4-12% acrylamide, Coomassie Brilliant Blue G250 stained**

**DUP sample 29**

1: Mr standard, 5 ul solution
2: UP<sub>sol</sub>1, ca. 11 ul extract
3: UP<sub>sol</sub>2a, ca. 11 ul extract
4: UP<sub>sol</sub>2b, ca. 11 ul extract
5: UP<sub>sol</sub>3, ca. 11 ul extract
6: DNAse I, 2 ug
7: UP<sub>sol</sub>5, ca. 6 ul extract

- Sample 29 was not aggregated or clumpy after resuspension in PBS. Proteomic analysis revealed the presence of *Bacteroides fragilis* with no evidence that the bacterium caused a urinary tract infection although some neutrophils were microscopically identified. The extraction experiments shown here were performed after a freeze/thaw cycle. The pellet of sample 29 was homogenized upon incubation with PBS and then PBS + 50 mM DTT (UP<sub>sol</sub>1 and UP<sub>sol</sub>2 fractions, respectively). The pellet derived from UP<sub>sol</sub>2 extraction was small. Incubation with DNAse I for 60 min, resulting in fraction UP<sub>sol</sub>3.

- In this experiment, the following extraction steps were equivalent to those described in Figure 1A, with the exception that the extraction step resulting in UP<sub>sol</sub>4 was not performed.

**Step 1:** extraction with PBS (UP<sub>sol</sub>1);
**Step 2:** two successive extraction steps with PBS + 50 mM DTT (UP<sub>sol</sub>2a and UP<sub>sol</sub>2b);
**Step 3:** incubation/extraction with PBS and DNAse I (UP<sub>sol</sub>3);
**Step 4:** incubation/heat extraction with denaturing solution (UP<sub>sol</sub>5);
Date: 05-24-16

NuPAGE gel, 4-12% acrylamide, Coomassie Brilliant Blue G250 stained

DUP sample 36

1: Mr standard, 5 ul solution
2: UP\textsubscript{sol}3, ca. 10 ul extract
3: UP\textsubscript{sol}2, ca. 10 ul extract
4: UP\textsubscript{sol}1, ca. 10 ul extract
5: UP\textsubscript{sol}5, ca. 10 ul extract
6: Bovine serum albumin, ca. 2 ug

Sample 20 was neither aggregated nor clumpy after resuspension in PBS. Proteomic analysis revealed presence of *Gardnerella vaginalis* with no evidence that the bacterium caused a urinary tract infection although some neutrophils were microscopically identified. The extraction experiments shown here were performed after a freeze/thaw cycle. The pellet of sample 36 was homogenized upon incubation with PBS and PBS + 50 mM DTT (UP\textsubscript{sol}1 and UP\textsubscript{sol}2 fractions, respectively). The pellet derived from the UP\textsubscript{sol}2 extraction step was small and was incubated with DNAse I for 60 min, resulting in the fraction UP\textsubscript{sol}3.

In this experiment, the following extraction steps were identical to those described in Figure 1A, with the exception that the extraction step resulting in UP\textsubscript{sol}4 was not performed.

Summary:
Step 1: extraction with PBS (UP\textsubscript{sol}1);
Step 2: extraction with PBS + 50 mM DTT (UP\textsubscript{sol}2);
Step 3: incubation/extraction with PBS and DNAse I (UP\textsubscript{sol}3);
Step 4: incubation/heat extraction with denaturing solution (UP\textsubscript{sol}5);

Date: 06-22-16

0.5% agarose gel, ethidium bromide stained

Sample 36

1: UP\textsubscript{sol}1, ca. 5 ul extract
2: UP\textsubscript{sol}2, ca. 5 ul extract
3: UP\textsubscript{sol}3, ca. 5 ul extract
4: UP\textsubscript{sol}5, ca. 5 ul extract
5: DNA standard, 1 ul solution

HBA/HBB: hemoglobin α and β
CK: cytokeratins
UMOD

(abbreviations also used in other images)
**Figure 2e**

Date: 12-19-13  
NuPAGE gel, 4-12% acrylamide, Coomassie Brilliant Blue G250 stained  
DUP sample 55

| 1: | Mr standard, 5 ul solution | 2: | UP\_sol 1a, ca. 11 ul extract | 3: | UP\_sol 1b, ca. 11 ul extract | 4: | UP\_sol 2a, ca. 11 ul extract | 5: | UP\_sol 2b, ca. 11 ul extract | 6: | UP\_sol 3, ca. 11 ul extract | 7: | DNAse I, 2 ug | 8: | UP\_sol 5, ca. 6 ul extract |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|

- Sample 55 was neither aggregated nor clumpy after resuspension in PBS. Proteomic analysis revealed presence of the uropathogen *Klebsiella pneumoniae* with no evidence that the bacterium elicited an immune response and urinary tract infection. Few neutrophils were microscopically identified. The extraction experiments shown here were performed after a freeze/thaw cycle. The pellet of sample 55 was homogenized upon incubation with PBS and PBS + 50 mM DTT (UP\_sol 1 and UP\_sol 2 fractions, respectively). The pellet derived from the UP\_sol 2 extraction step was small and was incubated with DNAse I for 60 min, resulting in the fraction UP\_sol 3.
- In this experiment, the following extraction steps were identical to those described in Figure 1A, with the exception that the extraction step resulting in UP\_sol 4 was not performed.

**Summary:**

Step 1: two extraction steps with PBS (UP\_sol 1a and UP\_sol 1b);  
Step 2: two extraction steps with PBS + 50 mM DTT (UP\_sol 2a and UP\_sol 2b);  
Step 3: incubation/extraction with PBS and DNAse I (UP\_sol 3);  
Step 4: incubation/heat extraction with denaturing solution (UP\_sol 5);
Sample 64 was derived from a urinary sediment that was neither aggregated nor clumpy after resuspension in PBS or PBS-DTT. The human subject had a urethral catheter-associated infection (CAUTI), and this sample was obtained after the catheter-associated biofilm dispersed into the urine sediment. Proteomic analysis revealed Proteus mirabilis as the infectious agent. The extraction experiments shown here were performed after a first freeze/thaw cycle. The pellet of sample 64 was homogenized upon incubation with PBS and PBS + 50 mM DTT (UPsol 1 and UPsol 2 fractions, respectively). The pellet derived from the UPsol 2 extraction was relatively large. Incubation with DNase I for 60 min did not change the pellet volume and resulted in fraction UPsol 3.

In this experiment, the following extraction steps were identical to those described in Figure 1A, with the exception that the extraction step resulting in UPsol 4 was not performed.

Summary:
Step 1: extraction step with PBS (UPsol 1a);
Step 2: two extraction steps with PBS + 50 mM DTT (UPsol 2a and UPsol 2b);
Step 3: incubation/extraction with PBS and DNAse I (UPsol 3);
Step 4: incubation/heat extraction with denaturing solution (UPsol 5);
Sample 88 was neither aggregated nor clumpy after resuspension in PBS. Proteomic analysis revealed presence of the vaginal commensals *Gardnerella vaginalis* and *Prevotella melanogenica* with no evidence that the bacterium elicited an immune response and urinary tract infection. Few neutrophils were microscopically identified.

The extraction experiments shown here were performed after a freeze/thaw cycle. The pellet of sample 88 was homogenized upon incubation with PBS and PBS + 50 mM DTT (UP\textsubscript{sol}1 and UP\textsubscript{sol}2 fractions, respectively). The pellet derived from the UP\textsubscript{sol}2 extraction step was small and was incubated with DNAse I for 60 min, resulting in the fraction UP\textsubscript{sol}3.

In this experiment, the following extraction steps were identical to those described in Figure 1A, with the exception that the extraction step resulting in UP\textsubscript{sol}4 was not performed.

Summary:
Step 1: extraction with PBS (UP\textsubscript{sol}1);
Step 2: extraction with PBS + 50 mM DTT (UP\textsubscript{sol}2);
Step 3: incubation/extraction with PBS and DNAse I (UP\textsubscript{sol}3);
Step 4: incubation/heat extraction with denaturing solution (UP\textsubscript{sol}5);
Sample 112 was viscous and clumpy. *In vitro* culture and proteomic analysis revealed *Staphylococcus aureus* as the infectious agent. The extractions related to this experiment were performed after a freeze/thaw cycle. The pellet of sample 112 remained clumpy and viscous and was not homogenized upon incubation with PBS and PBS + 50 mM DTT (UP_{sol}1 and UP_{sol}2 fractions, respectively).

- In this experiment, the following extraction steps were equivalent to those described in Figure 1A.

**Summary:**
Step 1: extraction with PBS (UP_{sol}1);
Step 2: two successive extraction steps with PBS + 50 mM DTT (UP_{sol}2a and UP_{sol}2b);
Step 3: incubation/extraction with PBS and DNAse I (UP_{sol}3);
Step 4: extraction/incubation with lysozyme and mutanolysin in the presence of the partially membrane-solubilizing reagents EDTA and CHAPS (UP_{sol}4);
Step 5: incubation/heat extraction with denaturing solution (UP_{sol}5);
Date: 04-30-15

NuPAGE gel, 4-12% acrylamide, Coomassie Brilliant Blue G250 stained

AUP sample 118

1: UP_{sol}1, ca. 10 ul extract
2: UP_{sol}2a, ca. 10 ul extract
3: UP_{sol}2b, ca. 10 ul extract
4: UP_{sol}3, ca. 10 ul extract
5: UP_{sol}5, ca. 10 ul extract
6: Mr standard, 5 ul solution

- Sample 118 was viscous and clumpy. \textit{In vitro} culture and proteomic analysis revealed \textit{Klebsiella pneumoniae} and \textit{Escherichia coli} as the infectious agents. The extractions related to this experiment were performed after a freeze/thaw cycle. The pellet of sample 118 became less clumpy and viscous but was not completely homogenized upon incubation with PBS and PBS + 50 mM DTT (UP_{sol}1 and UP_{sol}2 fractions, respectively). Homogenization was achieved with the DNAse I incubation step.
- In this experiment, the following extraction steps were equivalent to those described in Figure 1A, with the exception that the extraction step resulting in UP_{sol}4 was not performed.

Summary:
Step 1: extraction with PBS (UP_{sol}1);
Step 2: two extraction steps with PBS + 50 mM DTT (UP_{sol}2a and UP_{sol}2b);
Step 3: incubation/extraction with PBS and DNAse I (UP_{sol}3);
Step 4: incubation/heat extraction with denaturing solution (UP_{sol}5);
Date: 04-15-15

NuPAGE gel, 4-12% acrylamide, Coomassie Brilliant Blue G250 stained

AUP sample 94

1: UP$_{sol}$1, ca. 10 ul extract
2: UP$_{sol}$2, ca. 10 ul extract
3: UP$_{sol}$3, ca. 10 ul extract
4: UP$_{sol}$5, ca. 10 ul extract
5: M$_r$ standard, 10 ul solution

● Sample 94 was viscous and clumpy. *In vitro* culture and proteomic analysis revealed *Escherichia coli* as the infectious agent. The extractions related to this experiment were performed after a freeze/thaw cycle. The pellet of sample 94 remained clumpy and viscous and was not homogenized upon incubation with PBS and PBS + 50 mM DTT (UP$_{sol}$1 and UP$_{sol}$2 fractions, respectively).

● In this experiment, the following extraction steps were equivalent to those described in Figure 1A, with the exception that the extraction step resulting in UP$_{sol}$4 was not performed.

Summary:
Step 1: extraction with PBS (UP$_{sol}$1)
Step 2: extraction with PBS + 50 mM DTT (UP$_{sol}$2a);
Step 3: re-extraction with PBS + 50 mM DTT (UP$_{sol}$2b); gel lane not shown
Step 4: incubation/extraction with PBS and DNAse I (UP$_{sol}$3);
Step 5: incubation/heat extraction with denaturing solution (UP$_{sol}$5);

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Date: 04-27-15

0.5% agarose gel, ethidium bromide stained

Sample 94

left: 5: DNA standard, 1 ul solution
1: UP$_{sol}$1, ca. 1 ul extract
2: UP$_{sol}$2a, ca. 1 ul extract
3: UP$_{sol}$2b, ca. 1 ul extract
4: UP$_{sol}$3, ca. 1 ul extract
Figure 2k

Date: 02-13-15

NuPAGE gel, 4-12% acrylamide, Coomassie Brilliant Blue G250 stained

AUP sample 134

1: Mr standard, 5 ul solution
2: UPsol1, ca. 11 ul extract
3: UPsol2a, ca. 11 ul extract
4: UPsol2b, ca. 11 ul extract
5: UPsol3, ca. 11 ul extract
6: UPsol4, ca. 11 ul extract
7: UPsol5, ca. 11 ul extract

● Sample 134 was viscous and clumpy. In vitro culture and proteomic analysis revealed Klebsiella pneumoniae as the infectious agent. The extractions related to this experiment were performed after a freeze/thaw cycle. The pellet of sample 134 remained clumpy and viscous and was not homogenized upon incubation with PBS and PBS + 50 mM DTT (UPsol1 and UPsol2 fractions, respectively).

● In this experiment, the following extraction steps were equivalent to those described in Figure 1A.

Step 1: extraction with PBS (UPsol1);
Step 2: extraction with PBS + 50 mM DTT (UPsol2a);
Step 3: re-extraction with PBS + 50 mM DTT (UPsol2b);
Step 4: incubation/extraction with PBS and DNAse I (UPsol3);
Step 5: extraction/incubation with lysozyme, EDTA and CHAPS (UPsol4);
Step 6: incubation/heat extraction with denaturing solution (UPsol5);
nuPAGE gel, 4-12% acrylamide, Coomassie Brilliant Blue G250 stained
AUP sample 134
(sample was subjected to two freeze/thaw cycles prior to extraction of protein and DNA)

1: M_r standard, 5 ul solution
2: UP_{sol}1, ca. 11 ul extract
3: UP_{sol}2a, ca. 11 ul extract
4: UP_{sol}2b, ca. 11 ul extract
5: UP_{sol}3a, ca. 11 ul extract; 6: wash step with PBS, ca. 11 ul extract
7: UP_{sol}4, ca. 11 ul extract
8: UP_{sol}5, ca. 11 ul extract

● Sample 134 was viscous and clumpy. In vitro culture and proteomic analysis revealed *Klebsiella pneumoniae* as the infectious agent. The extractions related to this experiment were performed after two freeze/thaw cycles. The pellet of sample 134 became less clumpy and viscous but was not completely homogenized upon incubation with PBS and PBS + 50 mM DTT (UP_{sol}1 and UP_{sol}2 fractions, respectively). Homogenization was achieved with the DNAse I incubation step.

● In this experiment, the following extraction steps were equivalent to those described in Figure 1A, except that an additional wash step after DNAse I incubation was added.

Summary:
Step 1: extraction with PBS (UP_{sol}1);
Step 2: two extraction steps with PBS + 50 mM DTT (UP_{sol}2a and UP_{sol}2b);
Step 3: incubation/extraction with PBS and DNAse I (UP_{sol}3);
Step 4: pellet wash step with PBS;
Step 5: extraction/ incubation with lysozyme, EDTA and CHAPS (UP_{sol}4);
Step 6: incubation/heat extraction with denaturing solution (UP_{sol}5);
Date: 04-30-15
NuPAGE gel, 4-12% acrylamide, Coomassie Brilliant Blue G250 stained
AUP sample 20

1: M_r standard, 5 ul solution
2: UP_{sol}1, ca. 10 ul extract
3: UP_{sol}2, ca. 10 ul extract
4: UP_{sol}3, ca. 10 ul extract
5: UP_{sol}5, ca. 10 ul extract

- Sample 20 was originally viscous and clumpy. *In vitro* culture and proteomic analysis revealed *Proteus mirabilis* as the infectious agent. The extractions related to this experiment were performed after two freeze/thaw cycles. The pellet of sample 20 was less clumpy and viscous upon thawing for the 2nd time. It was homogenized upon incubation with PBS and PBS + 50 mM DTT (UP_{sol}1 and UP_{sol}2 fractions, respectively). DNA was released into the UP_{sol}1 and UP_{sol}2 fractions. No further homogenization was achieved with the DNAse I incubation step (UP_{sol}3 fraction). The DNA extraction was performed after a 3rd freeze-thaw cycle.

- In this experiment, the following extraction steps were equivalent to those described in Figure 1A, with that the extraction step resulting in UP_{sol}4 was not performed.

Summary:
Step 1: extraction with PBS (UP_{sol}1);
Step 2: extraction with PBS + 50 mM DTT (UP_{sol}2);
Step 3: incubation/extraction with PBS and DNAse I (UP_{sol}3);
Step 4: incubation/heat extraction with denaturing solution (UP_{sol}5);

Date: 10-28-15
0.5% agarose gel, ethidium bromide stained
Sample 20

1: UP_{sol}1, ca. 1 ul extract, after a 45 min incubation with DNAse I
2: UP_{sol}1, ca. 1 ul extract
right: 5: DNA standard, 1 ul solution
Date: 09-26-15
NuPAGE gel, 4-12% acrylamide, Coomassie Brilliant Blue G250 stained
AUP sample 122
(sample was not subjected to freeze/thaw cycle prior to extraction)

1: UP$_{sol}$1, ca. 10 ul extract
2: UP$_{sol}$2, ca. 10 ul extract
3: UP$_{sol}$3, ca. 10 ul extract
4: UP$_{sol}$5, ca. 10 ul extract
5: M$_r$ standard, 10 ul solution

● Sample 122 was viscous and clumpy. *In vitro* culture and proteomic analysis revealed *Candida albicans* as the infectious agent. The extractions related to this experiment were performed without freezing. The pellet of sample 122 remained clumpy and viscous and was not homogenized upon incubation with PBS and PBS + 50 mM DTT (UP$_{sol}$1 and UP$_{sol}$2 fractions, respectively).

● In this experiment, the following extraction steps were equivalent to those described in Figure 1A, with that the extraction step resulting in UP$_{sol}$4 was not performed.

Summary:
Step 1: extraction with PBS (UP$_{sol}$1);
Step 2: extraction with PBS + 50 mM DTT (UP$_{sol}$2);
Step 3: incubation/extraction with PBS and DNAse I (UP$_{sol}$3);
Step 4: incubation/heat extraction with denaturing solution (UP$_{sol}$5);
Date: 10-13-15
NuPAGE gel, 4-12% acrylamide, Coomassie Brilliant Blue G250 stained
AUP sample 124
(sample was not subjected to freeze/thaw cycle prior to extraction)

1: M_r standard, 5 ul solution
2: UP$_{sol}$1, ca. 15 ul extract
3: UP$_{sol}$2, ca. 15 ul extract
4: UP$_{sol}$3, ca. 15 ul extract
5: UP$_{sol}$4, ca. 15 ul extract
6: UP$_{sol}$5, ca. 15 ul extract

● Sample 124 was clumpy and had a very large volume. In vitro culture and microscopy revealed Candida albicans as the infectious agent. The extractions related to this experiment were performed without freezing. The pellet of sample 124 remained clumpy, but was partially homogenized upon incubation with PBS + 50 mM DTT (UP$_{sol}$1 and UP$_{sol}$2 fractions, respectively).
● In this experiment, the following extraction steps were equivalent to those described in Figure 1A.

Summary:
Step 1: extraction with PBS (UP$_{sol}$1)
Step 2: extraction with PBS + 50 mM DTT (UP$_{sol}$2a);
Step 3: incubation/extraction with PBS and DNAse I (UP$_{sol}$3);
Step 4: extraction/ incubation with lysozyme, EDTA and CHAPS (UP$_{sol}$4)
Step 6: incubation/heat extraction with denaturing solution (UP$_{sol}$5);

Figure 2o
Date: 06-22-16
0.5% agarose gel, ethidium bromide stained
Sample 124

1: DNA standard, 1 ul solution
2: UP$_{sol}$1, ca. 3 ul extract
3: UP$_{sol}$2, ca. 3 ul extract
4: UP$_{sol}$3, ca. 3 ul extract, incubation stopped with EDTA after 10 min
5: UP$_{sol}$3, ca. 3 ul extract, incubation stopped with EDTA after 60 min
UP sample 142 was not clumpy. In vitro culture and proteomic analysis revealed *Serratia marcescens* as the infectious agent. The extractions related to this experiment were performed from a UP sample obtained approximately 2-5 hours after urine specimen collection without cooling or freezing. The UP sample 142 became clumpy and aggregated after centrifugation and addition of PBS + 50 mM DTT. The aggregate was not homogenized upon incubation with PBS and PBS + 50 mM DTT (UP$_{sol1}$ and UP$_{sol2}$ fractions, respectively). The pellet derived from UP$_{sol2}$ extraction was incubated with DNAse I for 10 min, and then another 50 min, resulting in fraction UP$_{sol3}$.

In this experiment, the following extraction steps were identical to those described in Figure 1A, with the exception that the extraction step resulting in UP$_{sol4}$ was not performed.

**Summary:**
Step 1: extraction with PBS (UP$_{sol1}$);
Step 2: extraction with PBS + 50 mM DTT (UP$_{sol2}$);
Step 3: incubation/extraction with PBS and DNase I (UP$_{sol3}$);
Step 4: incubation/heat extraction with denaturing solution (UP$_{sol5}$);
Date: 07-19-16  
NuPAGE gel, 4-12% acrylamide, Coomassie Brilliant Blue G250 stained  
AUP sample 151  
(sample was not subjected to freeze/thaw cycle prior to extraction)

1: UP$_{sol1}$, ca. 10 ul extract  
2: UP$_{sol2}$, ca. 10 ul extract  
3: UP$_{sol3}$, ca. 10 ul extract  
4: UP$_{sol5}$, ca. 10 ul extract  
5: M$_r$ standard, 5 ul solution

Sample 151 was aggregated and clumpy. In vitro culture and proteomic analysis revealed *Escherichia coli* as the infectious agent. The extractions related to this experiment were performed from a UP sample obtained approximately 2-5 hours after urine specimen collection without cooling or freezing the neutrophils. The pellet of sample 151 remained clumpy and was not homogenized upon incubation with PBS and PBS + 50 mM DTT (UP$_{sol1}$ and UP$_{sol2}$ fractions, respectively). The pellet derived from UP$_{sol2}$ extraction was incubated with DNAse I for 60 min, resulting in fraction UP$_{sol3}$.

In this experiment, the following extraction steps were identical to those described in Figure 1A, with the exception that the extraction step resulting in UP$_{sol4}$ was not performed.

**Summary:**

Step 1: extraction with PBS (UP$_{sol1}$);  
Step 2: extraction with PBS + 50 mM DTT (UP$_{sol2}$);  
Step 3: incubation/extraction with PBS and DNAse I (UP$_{sol3}$);  
Step 4: incubation/heat extraction with denaturing solution (UP$_{sol5}$);

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Date: 07-15-16  
0.5% agarose gel, ethidium bromide stained  
Sample 151

1: DNA standard, 1 ul solution  
2: UP$_{sol1}$, ca. 3 ul extract  
3: UP$_{sol2}$, ca. 3 ul extract  
4: UP$_{sol3}$, ca. 3 ul extract, incubation stopped with EDTA after 10 min  
5: UP$_{sol3}$, ca. 3 ul extract, incubation stopped with EDTA after 60 min
NuPAGE gel, 4-12% acrylamide, Coomassie Brilliant Blue G250 stained  
AUP sample 157  
(sample was not subjected to freeze/thaw cycle prior to extraction)

1: Mr standard, 10 ul solution  
2: UP<sub>sol</sub>1, ca. 10 ul extract  
3: UP<sub>sol</sub>2, ca. 10 ul extract  
4: UP<sub>sol</sub>3, ca. 10 ul extract  
5: UP<sub>sol</sub>5, ca. 10 ul extract

Sample 157 was aggregated, moderately viscous and clumpy. In vitro culture and proteomic analysis revealed *Staphylococcus aureus* as the infectious agent. The extractions related to this experiment were performed from a UP sample obtained approximately 2-5 hours after urine specimen collection without cooling or freezing. The pellet of sample 157 remained clumpy and viscous and was not homogenized upon incubation with PBS and PBS + 50 mM DTT (UP<sub>sol</sub>1 and UP<sub>sol</sub>2 fractions, respectively). The pellet derived from UP<sub>sol</sub>2 extraction was incubated with DNase I for 60 min, resulting in fraction UP<sub>sol</sub>3.

In this experiment, the following extraction steps were identical to those described in Figure 1A, with the exception that the extraction step resulting in UP<sub>sol</sub>4 was not performed.

Summary:
Step 1: extraction with PBS (UP<sub>sol</sub>1);  
Step 2: extraction with PBS + 50 mM DTT (UP<sub>sol</sub>2);  
Step 3: incubation/extraction with PBS and DNase I (UP<sub>sol</sub>3);  
Step 4: incubation/heat extraction with denaturing solution (UP<sub>sol</sub>5);

Date: 07-15-16  
0.5% agarose gel, ethidium bromide stained  
Sample 157

1: DNA standard, 1 ul solution  
2: UP<sub>sol</sub>1, ca. 3 ul extract  
3: UP<sub>sol</sub>2, ca. 3 ul extract  
4: UP<sub>sol</sub>3, ca. 3 ul extract, incubation stopped with EDTA after 10 min  
5: UP<sub>sol</sub>3, ca. 3 ul extract, incubation stopped with EDTA after 60 min
Date: 07-07-16
NuPAGE gel, 4-12% acrylamide, Coomassie Brilliant Blue G250 stained
AUP sample 136

1: UP\textsubscript{sol}1, ca. 10 ul extract
2: UP\textsubscript{sol}2, ca. 10 ul extract
3: UP\textsubscript{sol}3, ca. 10 ul extract, 60 min DNase incubation
4: UP\textsubscript{sol}5, ca. 10 ul extract
5: Mr standard, 10 ul solution

- Sample 136 was aggregated and clumpy. In vitro culture and proteomic analysis revealed \textit{Escherichia coli} as the infectious agent. The extractions related to this experiment were performed from a UP sample obtained approximately 2-5 hours after urine specimen collection without cooling or freezing the neutrophils. The pellet of sample 136 remained clumpy and was not homogenized upon incubation with PBS and PBS + 50 mM DTT (UP\textsubscript{sol}1 and UP\textsubscript{sol}2 fractions, respectively). The pellet derived from UP\textsubscript{sol}2 extraction was incubated with DNase I for 60 min, resulting in fraction UP\textsubscript{sol}3.
- In this experiment, the following extraction steps were identical to those described in Figure 1A, with the exception that the extraction step resulting in UP\textsubscript{sol}4 was not performed.

Summary:
Step 1: extraction with PBS (UP\textsubscript{sol}1);
Step 2: extraction with PBS + 50 mM DTT (UP\textsubscript{sol}2);
Step 3: incubation/extraction with PBS and DNase I (UP\textsubscript{sol}3);
Step 4: incubation/heat extraction with denaturing solution (UP\textsubscript{sol}5);

Date: 07-15-16
0.5% agarose gel, ethidium bromide stained
UP sample 136

1: DNA standard, 1 ul solution
2: UP\textsubscript{sol}1, ca. 3 ul extract
3: UP\textsubscript{sol}2a, ca. 3 ul extract
4: UP\textsubscript{sol}2b, ca. 3 ul extract
5: UP\textsubscript{sol}3, ca. 3 ul extract, incubation stopped with EDTA after 60 min
Figure 2t

Date: 08-30-16
NuPAGE gel, 4-12% acrylamide, Coomassie Brilliant Blue G250 stained
AUP sample 146

1: UP_sol1, ca. 10 ul extract
2: UP_sol2, ca. 10 ul extract
3: UP_sol3, ca. 10 ul extract, 10 min DNAse incubation
4: UP_sol3, ca. 10 ul extract, 60 min DNAse incubation
5: UP_sol5, ca. 10 ul extract
6: M_r standard, 10 ul solution

● Sample 146 was aggregated and clumpy. In vitro culture and proteomic analysis revealed *Escherichia coli* as the infectious agent. The extractions related to this experiment were performed from a UP sample obtained approximately 2-5 hours after urine specimen collection without cooling or freezing the neutrophils. The pellet of sample 146 remained clumpy and was not homogenized upon incubation with PBS and PBS + 50 mM DTT (UP_sol1 and UP_sol2 fractions, respectively). The pellet derived from UP_sol2 extraction was incubated with DNAse I for 60 min, resulting in fraction UP_sol3.

● In this experiment, the following extraction steps were identical to those described in Figure 1A, with the exception that the extraction step resulting in UP_sol4 was not performed.

Summary:
Step 1: extraction with PBS (UP_sol1);
Step 2: extraction with PBS + 50 mM DTT (UP_sol2);
Step 3: incubation/extraction with PBS and DNAse I (UP_sol3);
Step 4: incubation/heat extraction with denaturing solution (UP_sol5);

Date: 07-15-16
0.5% agarose gel, ethidium bromide stained
Sample 146

1: DNA standard, 1 ul solution
2: UP_sol1, ca. 3 ul extract
3: UP_sol2a, ca. 3 ul extract
4: UP_sol2b, ca. 3 ul extract
5: UP_sol3, ca. 3 ul extract, incubation stopped with EDTA after 60 min
Figure 3. Western blot confirming the reactivity of S. aureus protein A (Spa) with anti-rabbit IgG HRP conjugate

Date: 08-30-16
NuPAGE gel, 4-12% acrylamide followed by western blot transfer to PVDF membrane

1: UP₃ from lysate of AUP sample 134, ca. 10 ul extract
2: Mr standard, 10 ul solution
3: recombinant S. aureus protein A (RPA-50, Cat. No. 1001-01, Repligen), 0.1 µg
4: lysostaphin digest of cell extract from S. aureus AUP sample #112 isolate, ca. 10 µg
5: lysostaphin digest of cell extract from S. aureus strain HIP5827, ca. 10 µg
6: UP₃ from lysate of AUP sample 112, ca. 10 ul extract

* The blot was performed using no primary antibody dilution (instead an incubation with 1% BSA in PBS-Tween 20, 0.05%, for 90 min was performed), followed by an incubation with a secondary antibody HRP conjugate (horseradish peroxidase conjugate of goat anti-rabbit IgG-HRP; Sta. Cruz Biotech, sc-2004) in a 1:10,000 dilution in 1% BSA in PBS-Tween 20, 0.05%, for 90 min and detection of protein recognition by 2nd antibody using the substrate 3,3',5,5'-Tetramethylbenzidine (KPL, Gaithersburg, MD; #54-11-50) in a colorimetric endpoint assay over 4 min.

Summary:
The reactivity of a major band at 50 kDa indicates that *S. aureus* protein A (Spa) from the recombinant protein (lane 3), two lysostaphin digests of *S. aureus* strains (lanes 4 and 5) and the UP₃ fraction of AUP sample #112 is recognized by the 2nd antibody-HRP conjugate. In conclusion, Spa from the clinical sample (AUP sample #112) actively binds IgG which is a relevant activity in the defense against immunoglobulins and opsonization.